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A Biochemical Study and Differentiation of Oral Bacteria, with Special Reference to Dental Caries

Dissertation
Submitted in partial Fulfilment of the Requirements
for the Degree of Doctor of Philosophy
in the Faculty of Pure Science
of Columbia University in
the City of New York

By
Israel J. Kligler, B.S., M.A.

New York City
1915

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PREFACE

The investigation reported in the succeeding pages was undertaken with the object of applying modern bacteriologic technique to the study of a field of bacteriology which, though the subject of numerous researches in the years immediately following Koch's epochal discoveries, has been practically neglected during the last fifteen years. That a revision of the subject of Oral Microorganisms was highly desirable is evidenced by the fact that, whereas the bacteria of the intestine are well known, none of the modern text-books presumes to state what organisms characterize the normal oral cavity, to say nothing of the diseased conditions.

Recently great impetus was given to studies of the microorganisms in Pyorrhœa, due to the importance attributed to that ailment as a focal point of origin of various systemic diseases. The bacteria of caries, however, have been left in the dark and totally ignored.

This study was started in order to obtain a comprehensive idea of the organisms prevalent in the deposits on normal as well as decayed teeth. The information obtained is by no means complete and many years of intensive study will undoubtedly be required to round out and perfect our knowledge of the oral microorganisms. This dissertation is merely the opening of a gate to a neglected field of research.

The man who deserves the credit for giving the impetus to, and being the guiding spirit in, this study (or rather series of studies) is Professor Gies. It is indeed a pleasure to acknowledge with thanks the stimulating suggestions and helpful guidance which he has given me in the progress of this work. I also wish to thank Professor C.-E. A. Winslow for much valuable criticism on the conduct of this work. Thanks are also due to all those who so willingly served as 'subjects' in these experiments.

I. J. K.

Biochemical Laboratory
Columbia University
December, 1915

K- 147

CONTENTS

		Page
I.	Historical (Reprinted from the *Journal of the Allied Dental Societies*, June, 1915, vol. x)	141–166
	1. Introduction	141
	2. Oral Microorganisms in General	143
	a Animal Parasites	144
	b Intermediate Forms	145
	c Bacteria	149
	3. Bacteria in Relation to Caries	160
II.	Experimental (Sec. One) (Reprinted from *Journal of the Allied Dental Societies*, September, 1915, vol. x)	282–330
	1. Plan or Work	282
	2. Methods	283
	3. Sources of Material	287
	4. Results	288
	a Quantitative Results	289
	b Qualitative Results	299
	5. Frequency of Occurrence and Abundance of the Various Types of Bacteria Under the Different Conditions Studied	305
	Correlation of the Data	311
	6. Description of Types	314
	Genus Streptococcus	314
	Genus Diplococcus	316
	Staphylococci	318
	Genus Bacillus	319
	Aerobic Spore Formers	320
	Anerobic Spore Formers (*B. putrificus*)	321
	Non Spore-Forming Rods	322
	Trichomycetes	325
	Cladothrix placoides	326
	L. buccalis	328

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Page

II. Experimental (Sec. Two). (Reprinted from the *Journal of the Allied Dental Societies*, December, 1915, vol. x) 445–458
7. Chemical Data 445
a Fermentation of Carbohydrates . . 446
b Effect of Mucinate on the Growth of oral Bacteria 448
c Action of oral Bacteria on salivary Mucinate 449
d Relative solvent Action of different Types of oral Bacteria, singly and in combination, on powdered Teeth in a Medium containing Carbohydrate 450

III. Summary of bacteriological and chemical Findings 453

IV. Concluding Discussion 455

V. Summary of general Conclusions 457

[Reprinted from THE JOURNAL OF THE ALLIED DENTAL SOCIETIES, 1915, pp. 141 to 166.]

CHEMICAL STUDIES OF THE RELATIONS OF ORAL MICROÖRGANISMS TO DENTAL CARIES[1]

BY WILLIAM J. GIES AND COLLABORATORS.

2. A Biochemical Study and Differentiation of Oral Bacteria with Special Reference to Dental Caries. (I)[2]

BY I. J. KLIGLER.

(From the Biochemical Laboratory of Columbia University, at the College of Physicians and Surgeons, New York.)

I. HISTORICAL.[3]

1. **Introduction.**—Early in the history of bacteriology, the flora of the oral cavity received special attention. It was soon realized by bacteriologists that in no other entrance to the body do more favorable conditions exist for the development of many types of microörganisms. That the mouth is a conspicuous port of entry for many infections has long been appreciated.

Mucus, epithelial fragments, toothpulp, various secretions and food rests provide an abundant variety of favorable media for bacterial growth in the mouth. (The contention of Sanarelli that salivary secretions possessed bactericidal properties has been disproven by Miller and others.) The border between the teeth and gums, the spaces between the teeth, and cavities in the teeth, offer diverse conditions of variable oxygen concentration, a very important determining factor in the environment of these delicate forms. The temperature, too, is very favorable for the development of most of the microörganisms, being, as shown by Bachelet, practically constant between 35.6° C. and 36.5° C. on the interior side of the teeth, and only slightly lower on the exterior side of the teeth.

Many observers have attempted to determine the nature of the flora of the human mouth. Numerous types have been isolated and described by various investigators without any attempt

[1] Reports of findings in investigations conducted under the auspices of the First District Dental Society of the State of New York.

[2] Accepted, by the executive officer of the Department of Biological Chemistry of Columbia University, as Part I of a dissertation, submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, in the Faculty of Pure Science, Columbia University, June, 1915.

[3] The second section of this dissertation will be published in the succeeding issue of the *Journal of the Allied Dental Societies.*

at systematic grouping. In many instances the descriptions of the types are so meagre that they are practically useless as a basis for comparisons of the available data. We cannot, at present, differentiate, except in a few instances, the passing invaders from the permanent inhabitants, or the harmless saprophytes from the true parasites, of the mouth. Of the forms described, there is little definite information regarding the abundance or frequency with which they occur in the mouth. Küster (Kolle and Wasserman, 1911) says in this connection: "As non-pathogenic cultivable bacteria such a large number have been described by various authors that it is out of the question to describe them fully here; aside from this, the reports regarding the frequency of occurrence, the morphology and biology, are unfortunately either incomplete or show such an inadequate bacteriological experience of the authors, that a comparison of their individual findings seems useless."

The same is also true of the bacteriology of normal and decayed teeth. The classic work of Miller did little more, unfortunately, than break the ground. The available technique in his day was so crude that it is surprising that Miller obtained the wealth of information he did. The greater number of characteristic mouth forms described by Miller fall into his group of "non-cultivable" types, his descriptions being purely morphological—obviously of little significance in the characterization of bacterial types.

Since Miller's day remarkable progress has been made in the development of bacteriological methods, in the perfection of a system of classification, and in the discovery of new tests for the differentiation of microörganisms by means of their biochemical activities. The purely morphological types of Miller's day are rapidly being replaced by more definite physiological species.

Although a worker here and there has applied the new methods for the isolation of a specific form found in or between the teeth, no attempt has been made to apply them to a comprehensive systematic study of the bacteria of healthy and diseased teeth. Probably the best summary of the subject from this standpoint, since Miller's, was that made by Goadby, who also contributed valuable information on this subject. Goadby's

work was done, however, more than fifteen years ago, his results are only qualitative, and his descriptions of characteristic types are meagre and generally based on the work of others. Rodella (1905) made an important contribution to this subject by calling attention to the anerobic flora of the saliva and its possible importance. As yet no one has confirmed Rodella's findings, nor has any one made a thorough study of the entire microbic life, aerobic and anerobic, the relation of the types to each other, their effects on one another and their *relative* abundance, frequency and importance.

In 1892 Miller wrote: "The great number of different bacteria which have been obtained in the mouth . . . has hitherto made their classification impossible. We are unable with few exceptions to state which of these bacteria occur most frequently in the mouth or under what conditions the different kinds develop best. . . . A classification of the bacteria of diseased tooth pulp has not been made as yet." This accurately indicates the condition of oral bacteriology at the present time.

2. **Oral Microörganisms in General.**—In speaking of the microbes of the mouth, we generally include all forms from all parts of the oral cavity. It is difficult to say with certainty, however, which of the organisms mentioned in the literature should be regarded as *inhabitants* of the mouth and which as visitors. Certain it is that much of the descriptive material is unreliable; but it is extremely hazardous in a summary, such as I am attempting to give here, to say with confidence which data can be relied upon. Certain criteria, somewhat arbitrary perhaps, had to be resorted to in selecting the designated types which probably make up the flora of the mouth. These criteria were: (a) the regularity with which the types are reported by the different workers; (b) the extent of the descriptions given; and (c) the frequency with which the types were found to appear in the mouth. In all cases attempts were made to discover from the literature the seat of the organism, its abundance in the mouth, and the frequency with which it occurs there. The forms regarding which there is uncertainty from these three standpoints, are excluded from consideration here.

Miller, in his classic work, divides the microörganisms of the mouth into two large groups: (1) the cultivable forms and

(2) the uncultivable forms. Into the latter group fall most of his characteristic buccal types. This division is, however, no longer tenable. Many of the uncultivable species reported by Miller have since been isolated. Besides, the division is purely artificial and should be replaced by a more natural one based on biologic relationships.

On a biologic basis the oral microörganisms fall into three main divisions: (1) Animal parasites: *protozoa;* (2) Intermediate forms: *spirochetes;* (3) Plant organisms: *bacteria.*

(1). ANIMAL PARASITES (PROTOZOA). The animal parasites have not been studied as intensively as bacteria, probably because of the greater difficulty involved. Only four types of protozoa supposed to *inhabit* the human mouth have been described. Of these four only one can be claimed with some *certainty* to be a specific mouth form. This is the *Endamoeba buccalis* (Prowazek).

Endamoeba buccalis is found exclusively in human beings and is present, according to Hartman, in almost every human mouth, being especially abundant in carious teeth. It is a small (6-32 μ), actively motile, ameba, forming only few pseudopodia. It shows, while at rest, a distinct division of ectoplasm and endoplasm, the former being more refractive and homogeneous, while the latter has many vacuoles. It has a small nucleus poor in chromatin material but readily visible. It divides in the usual way.

The same organism has probably been described by Grassi as *A. dentalis,* and by Gros as *A. gingivalis.* It is also quite likely that the ameba reported recently by Barrett to have been found in pyorrhea, and associated by him with the cause of the affection, is the same as *E. buccalis.* The description given by Barrett is very much like that for this form. The disintegrated white blood cells and bacteria in pyorrheal pockets offer ideal food for the development of these unicellular organisms. The relation of these protozoa to pyorrhea has not yet been determined, though Bass and John have been able to confirm the findings of Barrett and Smith. The rôle of the streptococci, and the favorable effects of autogenous vaccines, still remain to be explained. It may be, of course, that the cocci are secondary invaders and merely serve as food for the amebae; but this has not

yet been established. These findings show, however, that this type of protozoan is a usual *inhabitant* of the mouth.

Another ameba found several times in the human mouth, generally associated with osteomyelitis of the lower jaw-bone is the *E. Kartulisi* (Dœflein). This organism was discovered by Kartulis in Egypt, in 1893. It is highly pathogenic and is supposed to be localized in the mouth. It is often found there in connection with jaw-bone diseases. This ameba is 30-38 μ in diameter, shows no distinct differentiation between ectoplasm and endoplasm, and has a small nucleus containing a well defined nucleolus. It is actively motile and usually sends out only one long thin pseudopod.

Two other protozoa have been found, one in sputum, the other in tartar; but their descriptions are so inadequate that it cannot be determined to which group they belong. These forms are included here, however, because of the meagreness of the available data regarding mouth protozoa.

(a) *Protozoan* (Ellerman). A small motile form, very closely resembling cocci, except for their motility, measuring ½-1 μ. They are frequently found in the sputum. Ellerman observed them nine times in thirteen people.

(b) *Protozoan* (Baumgartner). Observed only in sections of teeth; generally present in the outer layer of the enamel. Two or three nuclei are generally present. (The lack of differential value of such a superficial description is obvious.)

(2) INTERMEDIATE FORMS (SPIROCHETES). The second or intermediate group of mouth organisms includes the spirochetes. These forms were first observed, in the mouth, by Leeuwenhoek (1722). Later one type was described by Miller (1892), under the name *Sp. denticola.* It was, however, one of the "uncultivable" forms and his description was necessarily superficial. Very little attention was paid to spirochetes until the epoch-making discovery by Schaudinn (1906) of the spirochete of syphilis (*Tr. pallidum*). This discovery gave a new impetus to the study of mouth spirochetes, and a number of renewed attempts were made to cultivate these organisms.

Mühleus, in 1906, reported success, and described a type of mouth spirochete, closely resembling the *Sp. denticola* of Miller, which grew anerobically on serum-agar. Hoffmann and Prowa-

zek (1906) made a careful study of a series of smear preparations and described three different types—(a) *Sp. dentium:* Delicate in structure; thin, flexible body; quite regular, closely set, shallow spirals; (b) *Sp. media:* Intermediate form; (c) *Sp. buccalis:* Large, thick spiral, with few flat curves. All had undulating membranes and flexible bodies and, therefore, belonged to the genus *Spirochaeta* (Ehrenberg).

Repaci (1912) reported the successful cultivation of four mouth spirochetes in glucose-agar under anerobic conditions. He gives a thorough description of the biological characters of these forms and claims that they are different from the Prowazek types. An analysis of his descriptions, however, brings his types A and C into the intermediate group (*Sp. media*); his type B into the *Sp. buccalis* group, while his type D remains unclassified.

Noguchi (1912) cultivated successfully, by his own method, two spirochetes from teeth which belonged to the *Sp. dentium* and *Sp. media* groups, respectively, and suggested the names *Treponema microdentium* for the former and *Tr. macrodentium* for the latter. Later in the same year (1912) he reported the isolation of another type from a case of *pyorrhoea alveolaris,* which is smaller than *Sp. dentium* but resembles it closely, except for the fact that it produces in pure culture both mucin and a strong fetid odor. This he names *Treponema mucosum.*

Gerber, after a series of smear studies (1910, 1912), described at least five types which he claims are normally present in the mouth. His results can not, however, be accepted with the same confidence as those of Mühleus, Repaci and Noguchi (especially the last two, who worked with pure cultures). The species described by the latter three observers are, therefore, the only ones which may be tentatively accepted as distinct types.

According to Noguchi (1912), the relative number of the three types that occur in the normal mouth varies "greatly according to the conditions of the mouth and to the localities from which the material is obtained." The smallest type is more abundant between the teeth and gums, and in the cavities of carious teeth. The remaining two types, on the other hand, are more frequently found in the mucus about the tonsils and pharynx, and in large numbers in ulcerative stomatitis.

Gerber (1910) found spirochetes in normal and diseased

mouths, and especially on tartar in normal mouths. The following table given by him is of interest:

			Spirochetes.	
Normal Mouth.	No. of Examinations.	Cases.	+	—
Tonsils	39	38	10	29
Tartar	35	34	29	6
Tongue	8	6	4	4
Miscellaneous	7	5	0	7
Diseased Mouth.				
Tonsils	28	22	7	21
Carious teeth	2	2	2	0
Tongue	2	2	1	1
Miscellaneous	21	12	7	14

It is evident that these forms are not more prevalent in diseased mouths than in healthy ones; and it is of especial interest to note their predominance in tartar. Unfortunately, Gerber has not examined a sufficiently large number of carious teeth, nor does he tell us how abundant were the spirochetes in the various cases examined.

Thibandeau examined the secretions between the teeth and gums, and the mucus on the papillae of the tongue, in 149 healthy people and reports the presence of one or another type of spirochete in 41 per cent. of the cases. He, also, fails to give us data regarding the relative abundance of the organisms. It must also be borne in mind that both authors worked with smears only. It is evident from these findings that spirochetes are usual inhabitants of normal mouths.

Other workers, notably Plaut and Vincent, Goadby, and Weaver and Tunnicliff, observed spirochetes in association with other forms, especially the *B. fusiformis,* but the first were unsuccessful in cultivating them while the last obtained them in impure cultures. Tunnicliff even claimed that the spirochetes and fusiform bacilli were morphological variations of the same organism, a view that has not been confirmed. Goadby confuses spirillae with spirochetes, and one cannot be certain to which forms he alludes in his discussions. He claims that " presence of spirilla is a marked feature of pathological conditions," and that he found them in 75 out of 85 cases of chronic alveolar ostitis, but it is doubtful whether he refers to spirochetes or to spirillae.

Before proceeding to describe the types that occur most frequently in the mouth, a word may be said regarding the system-

atic position of the *Spirochaetes*. There has been a good deal of discussion as to whether these organisms are protozoa or bacteria. Many protozoölogists agree with men like Prowazek, Doeflein and others, on one side, claiming that they are protozoa; or with Novy and Dobell, on the other, contending that they are true bacteria. The arguments, pros and cons, need not be repeated here. The fact is that the spirochetes resemble both bacteria and protozoa, and, for the present at least in accordance with the position taken by Calkins and others, should be considered as an intermediate group.

The mouth forms belong to the genus *Spirochaeta* (Ehrenberg) or to *Treponema* (Schaudin) according as they are parasitic or not parasitic. Since the types found in the mouth have not been observed elsewhere, we may follow the lead of Noguchi, and include them under the genus *Treponema*. Exception must, however, be taken to the specific names suggested by Noguchi, as the names *dentium* and *media* take precedence over his *microdentium* and *macrodentium*, respectively. The three types of spirochetes which occur frequently in the human mouth are, therefore: (a) *Treponema dentium* (Koch); (b) *Treponema medium* (Hoffman and Prowazek); (c) *Treponema buccalis* (Cohn). The descriptions which follow are taken from those given by Mühleus, Repaci and Noguchi.

Treponema dentium (Koch, Noguchi). Morphology: A small, delicate, flexible form, 4-10 μ long, about 0.2 μ thick; regular, closely set, shallow spirals, on an average 14 in number; the ends taper to fine points. Motility: Rotating, active. Cultivation: Mühleus cultivated what was probably a mixed culture of the dentium and media types on horse serum-agar (1-3) under strictly anerobic conditions; Repaci reports successful isolation in glucose-agar (this probably refers only to the *Sp. media* and *Sp. buccalis* types); Noguchi used the serum-agar-tissue medium employed by him for the isolation of *Tr. pallidum;* strict anerobic conditions are absolutely essential. Cultural characters—Serum-agar: Growth becomes perceptible between the fifth and tenth days at 37° C. only; along the stab canal the growth appears as an irregular whitish streak without definite contour; young cultures have *slight* odor but, after about three weeks, a characteristic fetid odor is observed. Broth: Forms a flaky dirty sedi-

ment leaving the broth clear; gives a marked fetid odor; does not produce gas. Pathogenesis: No pathogenic effect observed in rabbits, guinea pigs and mice. Occurrence: At juncture between teeth and gums, and in carious teeth.

Treponema medium (Hoffman and Prowazek). Morphology: Varies with age and condition of culture; in young culture they are plump, short, with rather irregular shallow curves, the extremities taper abruptly, show double refraction; in older cultures they are longer and thinner, and taper more gradually; the curves are shallow, regular and almost rectangular; the organism is 0.7-1.0 μ thick and 3-8 μ long with 2 to 8 curves in young culture, and 0.3 μ wide and 12 μ long with 14 or more curves in older cultures. Cultivation: It is strictly anerobic and grows only at 37° C.; glucose-agar, serum-agar, or serum-tissue-agar can be used for isolation; growth appears in 5-10 days, not earlier. Cultural characters—Serum-agar: faint, hazy, almost transparent colonies.—Liquid media: grows only feebly in liquid media; does not attack glucose, dextrin or sucrose; slight action on lactose; milk slowly acidified; no gas, slight odor. Pathogenicity: Not pathogenic for laboratory animals. Occurrence—In mucus on teeth, tonsils and pharynx and, in large numbers, in ulcerative stomatitis.

Treponema buccalis (Cohn). Morphology: Large mouth spirochete, ½-1 μ thick, 12-20 μ long; the spirals are polymorphic, showing many S forms, with flat large uneven curves; ends are rounded. Motility: Marked. Staining: Stains well with Giemsa and Burri stain; stains unevenly with gentian violet and other anilin dyes. Cultivation: Repaci is the only one who apparently succeeded thus far in isolating and growing this type in pure cultures in glucose-agar under strictly anerobic conditions; growth appears after the third day; grows only at 37° C. Cultural characters—Glucose-agar: Regular, glistening, discoid colony; whitish at edge, yellowish in center; cultures give putrefactive odor. Pathogenicity: Slightly pathogenic for rabbit and guinea pig.

(3). PLANT ORGANISMS (BACTERIA). By far the largest group of organisms found in the normal mouth belong to the order *Schizomycetes*. All the three main families—*Bacteriacae, Coccaceae* and *Spirillaceae*—as well as some of the higher bac-

teria—*Leptothrix, Sporothrix*—are always well represented. Here, especially, great confusion exists, since many cultivable forms have been isolated and separately described by different workers; and it is extremely difficult to differentiate between permanent mouth parasites and transient forms. The mouth is so veritably a gateway for all forms of bacteria that it is not sufficient merely to record the fact that such and such organisms have been found there. Systematic comparative studies must be undertaken to determine the relative abundance and frequency of occurrence of the types detected.

The first extensive study was made by Miller (1892) who, in his classic book, laid the foundation of dental bacteriology. He divides the mouth bacteria into (a) specific mouth forms, and (b) cultivable non-pathogenic and chromogenic types. The cultivable types, however, were most likely transient air, water and food types, while the specific mouth forms were not cultivable on the media then in vogue. Furthermore, Miller, working on the assumption that anerobes could not exist in the mouth, did not attempt to cultivate any anerobic bacteria!

Besides this classic contribution, a few others stand out from among a host of publications. Podbielsky (1891) examined the mixed sputum, and teeth and tongue scrapings, of fifty healthy individuals—25 adults and 25 children. He found *Sp. buccalis* absent from only 9 children between 5-14 months of age; while *Lept. buccalis* was absent from only 11 children less than 7 years of age. Comma forms were observed in 26 and rods in 15 cases. He observed a tetracoccus in 20 cases. He records the cultivation of 12 types of liquefying and 3 of non-liquefying cocci; 7 liquefying and 9 non-liquefying bacilli; 2 vibrios, 1 cladothrix and 2 yeasts. Though his work seems to be painstaking, there is no record of the frequency in occurrence of these types except in the two instances mentioned. His descriptions also fail to accord with any definite system.

The significant point in Podbielsky's examinations is the comparative absence of spirochetes and leptothrix forms in young children. This observation was confirmed by Oshima (1912), who found leptothrix absent from toothless infants but increased in abundance with the advancing age of children. Oshima also found that cocci occur abundantly at all ages, gen-

erally predominating over all other types. Oshima's results are also of interest in that he studied the flora of the mouths of 200 *normal* children from the ages of 1 month to 8 years. Unfortunately he examined smear preparations only, and hence his results merely indicate the predominance of certain large groups. His general results are tabulated below, though the crude method he employed renders their value doubtful.

Age	*No. of Cases*	*Types*	*No.*
6–12 mos.	78	Cocci	all
		Leptothrix	few
2 years	47	Cocci	all
		Leptothrix	18
3 years	18	Cocci	18
		Leptothrix	15
		Spirillae	12
4 years	12	Cocci	all
		Leptothrix	all
		Spirillae	all
5 years	11	Cocci	9
		Leptothrix	11
		Spirillae	11

The condition of the mouths of children 6, 7 and 8 years of age was similar to that found in children of 5.

Other studies bearing on this subject are those of Freund (on the mouth chromogens), Vignal, Vincentini, Sieberth, Williams, Dobrzyniecki and Goadby. The best work along this line was done by the last named author, who made a comparatively thorough study of the bacteria of dental caries and also succeeded in cultivating two of the specific mouth forms mentioned by Miller. Rodella's work on the mouth anerobes has already been referred to.

An interesting contribution to this subject has recently been made by Pickerill and Champtaloup, who studied the bacteria of the mouths of Maori children 4-15 years of age. Fifty individuals immune to caries were examined, Gram stains having been made of mixed saliva and of gingival deposit. The value of this work, like that of Oshima, is greatly impaired by failure to carry out exact quantitative cultivation. Their results have, however, a general interest in showing the nature of the predominant types in these normal, immune children; and the difference between the flora of the saliva and that of the gingival deposit.

		Cocci			Bacilli				Threads		Spirals	
Organism	Gram Negative.	Strep.	Staph.	Dip.	Maximus	Other Forms	Mesentericus	Fusiform	Leptothrix	Fine Threads	Commas	Spirochetes (?)
Saliva: per cent...	79	98	37	42	55	26	61	20	45	29	22	39
Gingival deposit: per cent.....	88	88	34	42	54	32	54	32	68	54	74	80

It is evident, from what has been said, that our knowledge of the flora of the mouth is not in a very satisfactory condition. I shall attempt, however, in this section as in the previous ones, to indicate the probable nature of the mouth bacteria, their characters and abundance, by a critical analysis of the data at hand.

Bacilli of the Normal Mouth. A large number of rod-shaped organisms have been found frequently in the normal mouth. Such forms as *B. diphtheriae, B. pseudodiphtheriae, B. coli,* and others have been found by numerous investigators in normal, healthy mouths. These cannot, however, be considered characteristic mouth bacteria. A number of rod forms are practically always found in the mouths of all normal people. These may be divided into aerobic and anerobic groups.

(a) *Aerobic bacilli.* There is only one organism specific for the mouth that belongs to this group. This is the *B. maximus buccalis* described by Miller and found by him quite frequently in the normal and diseased mouth. It was first isolated and thoroughly described by Goadby under the name of *B. maximus.* Goadby believes that it is identical with both Miller's and Vignal's *Leptothrix buccalis.* From Goadby's description it is undoubtedly a long bacillus having a tendency to form filaments. In older cultures spores may be observed. This form has been isolated by him from a number of cases and carefully described.

Another aerobic rod characteristic of the mouth has been isolated and described by Baumgartner under the name *B. iogenum,* which the author believes to be identical with the *Jodococcus vaginatus* described by Miller. The description of the bacillus is briefly as follows: *B. iogenum*—Morphology: 0.8-1.7 μ x 5-25 μ; stains unevenly with iodin, showing round, coccus-

like, blue granules in an almost colorless sheath (these granules are, according to Baumgartner, the cocci described by Miller). Biology: The bacillus is readily isolated on ascitic-fluid-, serum- or sputum-agar; is strictly aerobic and fails to liquefy gelatin. No data are given as to spore formation, staining reactions, growth on ordinary media, acid production in sugar broth, indol production, etc. I am not at all convinced that the author was dealing with a bacillus and not with a member of the sporothrix group of organisms. I believe it was the latter.

Aside from these strictly oral rods, a number of others are invariably found which belong mainly to the group of air and water saprophytes. Thus, Goadby found different varieties of *B. mesentericus and B. proteus.* Vignal mentions a number of different types of bacilli, but his descriptions are so poor that they are practically useless. Pigment-producing bacilli have also been isolated from the mouth by Dobrzyniecki, Freund and others; but, though the descriptions and data as to abundance and frequency are meagre, the forms undoubtedly belong to the group of saprophytic chromogens. Red, yellow and green pigment-producers are also described by Miller. These are most likely the prodigiosus, lutea and fluorescens types, respectively, found abundantly in air, water, etc.

(b) *Anerobic bacilli.* It may appear very improbable, but it nevertheless is true, that up to 1905, when Rodella published his paper on the anerobes in the mouth, no mention was made of these forms by any of the workers in this field of bacteriology. Neither Miller nor Goadby, nor the summary in Scheff's Handbuch, alludes to these microbes. Among the various anerobic forms that aroused the greatest interest and stimulated most work by various investigators, are the fusiform bacilli. These were first described by Plant (1894) and by Vincent (1896) in association with spirochetes in certain pathological conditions now known under the name Vincent's angina. Numerous other investigators afterwards observed and studied these organisms, and attempted their isolation; but it was not until 1904 that Lewkowicz reported successful results.

The fusiform bacilli occur almost invariably in the normal mouth, and are especially abundant in the various mouth diseases.

Krumwiede isolated these organisms from noma, ulcerous tongue, angina, pyorrhea, carious teeth, and spongy bleeding gums. Ozaki isolated them from tartar of healthy people. Repaci found them in the mouth of an individual in good health. Rodella claims that they are sometimes present in large numbers on the tartar on healthy teeth.

Besides the papers mentioned above, there is an enormous literature, consisting mainly of clinical reports on the presence of these types in various pathological conditions. An idea of the size of this literature may be obtained from the fact that, in 1904, Beitzke summarized 113 papers not including Italian, and in 1907 Babes (Kolle and Wassermann) in his article on the fusiform bacilli, also listed a large number of papers. From all this mass, however, no definite positive conclusion can be drawn, since only a few workers succeeded in cultivating these bacteria; and the descriptions are based, in each case, on only one or two strains, and disagree in many particulars.

Soon after Lewkowicz's report of the successful isolation of fusiform bacilli, Ellerman (1905) and Mühleus and Hartman (1906) succeeded in cultivating them from a case of gangrenous stomatitis, and from normal mouths, respectively. In 1908 Baumgartner found fusiform bacilli in gangrenous tooth pulp and in the deep layers of carious teeth, but did not grow them in pure culture. Fairly detailed descriptions of these bacteria were published by Repaci (1909) and Ozaki (1912), who succeeded in cultivating them in pure cultures from normal mouths. Rodella (1905) also reports the isolation of a fusiform bacillus from carious teeth but curiously enough, he reports the presence of spores which were not observed by any other worker. It is more than likely that either these spores were contaminations or else he was not dealing with fusiform bacilli.

The only systematic study of a large number of pure strains of fusiform bacilli obtained from different sources is that reported by Krumwiede and Pratt (1912).

While there are a few points, in this connection, on which all authors agree, the greatest possible confusion exists regarding other properties. All agree that the organism grows only under anerobic conditions, that it is Gram-negative (except Ro-

della), that it stains unevenly, that it is non-spore-bearing (except Rodella), that it has pointed ends and, finally, that it shows a certain pleomorphism. On the other hand, Lewkowicz, Ellerman, Mühleus and Hartman, and Weaver and Tunnicliff all claim that the organism grows only in the presence of serum; while Repaci and Ozaki claim that serum is not necessary. Krumwiede and Pratt state that serum is necessary for successful isolation, that after isolation the organism will grow for a while without serum, but that for continued vigorous growth serum is necessary. Evidently different strains vary in this respect.

More decided differences exist regarding pathogenicity and fermentation reaction. Repaci's strain attacked glucose, lactose and sucrose, and was highly pathogenic. Ozaki's strain attacked none of the sugars and, like the strains reported by the other workers, was non-pathogenic. Krumwiede's strains, 15 in number, all fermented glucose, failed to ferment lactose, while about half attacked sucrose.

It is possible, of course, that this group of organisms consists of more than one species and that there are saprophytic as well as parasitic forms. For the present, however, until more exhaustive study is made, we can only accept the results obtained by Krumwiede and Pratt as of definite value, since they are the only authors who worked with a number of strains of different origins. The following description is taken largely from the report of these authors.

B. fusiformis. Morphology: The typical organism is a double pointed bacillus, straight or somewhat bent, granular and very variable, varying with the medium; in solid media the organism is uniform; in liquid media filaments are generally formed; does not produce spores. Motility: The organism is not motile. Gram-stain: negative. Characteristics of growth—Agar: Grayish white colonies, which, in shake-cultures, have a puff-ball appearance.—Broth: Flocculent growth in bottom of the tube. Indol production: Always marked. Odor: All cultures give a disagreeable fetid odor; hydrogen sulfid has been observed by most workers. Fermentation: Very likely fermenting and non-fermenting strains exist; Ozaki's strain did not ferment any sugars; Repaci's fermented glucose, lactose and sucrose; Krum-

wiede's strains all fermented glucose, galactose, fructose; while some also fermented sucrose; all failed to attack arabinose, lactose, raffinose, dextrin, maltose, dulcite, inulin and glycerol; no gas was produced in any of the sugars. Viability: The culture may remain alive from 20-60 days depending on where it is kept; longer in the ice box than in the incubator. Pathogenicity: Unfortunately Krumwiede and Pratt did not determine the pathogenicity of their strains; of the other workers, Repaci is the only one whose strain was highly pathogenic; Paul, working with a mixed culture of fusiform bacilli and cocci, isolated from a case of gingivitis, found that it produced subcutaneous abscesses and caused death in three days when injected intraperitoneally; of course, the coccus may have played a more important rôle in this than the fusiform bacillus.

Besides this form, which is undoubtedly an inhabitant of the mouth, Rodella would include the well-known anerobes belonging to the *B. putrificus* and *B. butyricus* groups, respectively, which possess powerful proteolytic properties, as well as the power of splitting carbohydrates to butyric acid. So far, however, his work has not been confirmed, while Baumgartner (1913) maintains that he was unable to demonstrate the presence of anerobes in carious teeth.

Cocci of the Normal Mouth. Members of the diplococcus, streptococcus and staphylococcus groups are always present in the normal mouth. Pasteur, Sternberg, and more recently Park and Williams, and others, have isolated typical pneumococci from saliva, mouth, nose and throat. *Diplococcus catarrhalis* has been found in sputum by Seifert, Pfeiffer and numerous other workers. Other varieties of diplococci are found, but these have not been definitely described.

Streptococci are always abundant in the mouth. In fact they are so numerous that, when sputum is inoculated into sugar broth, they so rapidly overgrow all other types as to appear almost in pure culture. Goadby, following Lingelsheim's nomenclature, designates what he calls the mouth streptococcus as *Str. brevis*. The classification of this group of bacteria is, however, in an uncertain state, while it has been shown by many observers (Hopkins, Kligler and others) that length of chain is not a satisfactory criterion for the differentiation of these organisms.

Staphylococci of the white and orange varieties have been found frequently by Goadby, Pobiedoniesz, Dobrzyniecki and others. But these forms are found with equal, or greater frequency on the surface of the skin and other parts of the body. It is possible that there are specific mouth varieties, but these have not been described. The *St. tetragenus* (*Alb. tetragenus*), discovered by Gaffky, has been frequently found in the healthy mouth as well as in abscesses.

A coccus supposed to be specific for the mouth, characterized by its blue colorization with iodin, was described by Miller under the name *Jodococcus vaginatus*. No other reference was made to this organism but, recently, Baumgartner claimed that it was identical with his *B. iogenum*. Nothing definite can be gathered from the meagre descriptions at hand.

Anerobic cocci have been reported by Baumgartner and by Ozaki, the former from a fetid abscess, the latter from the mouth of a healthy individual. Ozaki records the property of gas production in sugar media, a character not as yet known to exist among the cocci. Since these are isolated reports of single doubtful findings it is hardly desirable to give a more detailed description of these types.

Spirilla in the Normal Mouth. Miller described a spirillum, which he found in each mouth in varying proportions depending on the care given it. It occurs in small numbers in "clean" mouths and very abundantly in "neglected" mouths. This vibrio he named *Spirillum sputigenum*. It is comma-shaped, actively motile, and closely resembles the cholera spirillum. Miller did not succeed in cultivating this form on any of the media tried by him.

Goadby and Mühleus both cultivated vibrios, the former an aerobe, the latter a strict anerobe, which each claimed to be identical with the *Sp. sputigenum* of Miller. Which one of these authors is right is hard to tell. The mouth harbors at times a number of vibrios, Miller himself having isolated three aerobic types. It is likely, therefore, that the anerobic comma-shaped organism isolated by Mühleus is the true *Sp. sputigenum*. Repaci succeeded in isolating three anerobic spirals, two from a case of leucoplacia and one from a normal mouth. Those isolated from

the pathological condition were motile and pathogenic, the other was non-motile and non-pathogenic. All attacked glucose, failed to liquefy gelatin, and did not produce indol. The organism isolated by Mühleus was like that of Miller: actively motile and somewhat larger than *Sp. cholerae.* No further description was given. The spirillum isolated by Goadby was very fully described by him. In general it resembled the other cholera-like vibrios.

It is evident, from the above, that a number of vibrios (both aerobic and anerobic) occasionally occur in the mouth, but as yet little more than the fact that someone has succeeded in isolating them is definitely known. The description of the spirillum isolated by Mühleus, for example, is so meagre and can so readily be applied to any comma-form, that it is impossible to compare his form with those reported by Repaci. These organisms are at best so difficult to differentiate that they must be studied carefully and systematically, and only a series of such investigations will ultimately prove of value in determining the microbic conditions of the mouth cavity and the teeth proper. It is not altogether improbable that the so-called mouth spiral form is not specific for the mouth but is derived from water.

Trichomycetes of the Normal Mouth. This group of higher bacteria seems to be especially characteristic of the mouth, and the abundance of its representatives is a fair index of the condition in which the teeth are kept. Pobiedoniesz and Oshima found them in every mouth except those of toothless infants. Pickerill reports leptothrices in 68 per cent. of the cases examined. Other observers, notably Miller, Vincentini, Goadby, and Williams, have found them in normal mouths. Miller described two types which he classes in the genus *Leptothrix* but neither of which he was able to cultivate. Vincentini reports another variety of *Leptothrix,* also uncultivated, while Dobrzyniecki reports the cultivation of still another type of *Leptothrix.* In fact, any thread like form observed in the mouth has been classed in the genus *Leptothrix,* so long as no branching was noted. The classification of this group is in a very unsatisfactory state, and it is quite obvious that we must adopt a common tongue if we are not to be drowned in a Babel of names all representing the

same thing. At the laboratory of the American Museum of Natural History the author has had the singular experience of obtaining the same organism under three different generic names —*Actinomyces, Streptothrix* and *Sporothrix;* while apparently different organisms came under the same generic name. It is quite likely that, following the lead of Miller, thread forms that were not *Leptothrix* were for convenience classed under that name.

Probably the best classification suggested thus far is that by Jordan, which follows:

Trichomycetes
- *Leptothrix*—No branching.
- *Cladothrix*—" False " branching.
- *Nocardia* (*Streptothrix*)—
  True branching; spores.
- *Actinomyces*—
  True branching; no spores.

Dobrzyniecki was apparently the only one who succeeded once in growing in pure state a culture of *Leptothrix,* which he isolated from a root filling. He named this strain *Leptothrix placoides alba,* and described it as follows:

Leptothrix placoides alba. Morphology: Chains of bacilli forming tangled threads, showing also coccoidal and bacillary forms; stains by Gram and with ordinary dyes. Biological Characters: Aerobic, liquefying, non-motile bacillus. No spores observed. Characteristics of growth—Agar-streak: Raised, clear, cartilaginous mass of isolated colonies in 48 hours; in 8-10 days they flow together and may be lifted with platinum needle. Gelatin plate: in 48 hours, minute, raised, whilte colonies composed of masses of threads; in three days the gelatin is liquefied.

The other forms reported are *L. innominata,* by Miller; *L. racemosa,* by Vincentini; and *L. buccalis,* by Vignal. They have been observed merely in stain preparations and may or may not be true leptothrices. The L. *buccalis* of Vignal appears, from the meagre description, to resemble the *B. buccalis* (Miller). There is nothing to prove the contrary, since these bacteria are highly pleomorphic and may readily present different appearances under diverse conditions. *The only criterion for the proper*

differentiation of types is the isolation and study of the morphological and biochemical characters of the organisms.

Miller also reported a type which he named *Strept. buccalis,* which was isolated and described by Goadby. Since a complete description is given by him it is hardly necessary to repeat it here.

From the summary presented above it is evident that our knowledge of the microörganisms of the mouth is not based on thorough systematic investigation, but on isolated reports by a number of individuals interested rather in some specific types of bacteria than in the flora of the mouth as a whole. As a result, our knowledge of the relationship of the various types found, their importance and significance, is vague and incomplete. We know a great deal about some forms, such as the fusiforms, and exceedingly little about others. A considerable amount of work is necessary to determine the nature, relationship and relative abundance of the different organisms in the normal and diseased buccal cavity, before we can undertake an exhaustive study of the rôle of these invaders, singly and in combination, in the disturbances that result from their activities.

3. **Bacteria related to Dental Caries.**—Theories of disease and its causation are generally affected by the predominant mode of thought at the time they are formulated. This general principle is well illustrated in the case of dental caries. The main theories purporting to explain the etiology of this affection fall into definite chronological periods. Thus, first came the "stagnation" theory, dating back to the 5th century B. C. and Hippocrates, and still finding vogue in the middle of the 18th century (Bourdet, Bell). Then came the phenomenal development of the science of chemistry at the end of the 18th and the beginning of the 19th century, and Pasch, Becker, Ringelman and others brought forward the purely chemical theory of caries, which found wide acceptance at the time. Caries was considered to be a chemical decomposition of the teeth by means of acids formed in the destruction of food particles.

This was followed by the physiological theory of "inflammation," promulgated by Thomas Bell in 1835 and later (1886) by Heitzman and Boedecker. "There occurs a primary inflammation in dentin . . . running its course, . . . and leading, as all inflammatory processes do, either to a new forma-

tion or destruction by suppuration. Inflammation causes first a solution of the lime salts and then a liquefaction of the basic substances."

With the discovery of parasites as causal agents of disease, in the middle of the last century, Klencke (1850) came forward with a report that he discovered a protococcus which liquefied dentin and which he considered the cause of caries. Then came the epochal discoveries of Pasteur. Following in his footsteps, Leber and Rottenstein (1867) formulated the chemico-parasitic theory of caries; and were followed by Milles and Underwood who, for the first time, presented bacteriological proof to substantiate this theory.

The man who did most in support of the parasitic theory of caries, and at the same time dealt a death blow to the other theories, was Miller. A pupil of Koch, and stimulated by the wonderful discoveries of his master, Miller conducted an extensive series of investigations on the bacteriology of the mouth in general and of caries in particular, and became the "father" of dental bacteriology. The theory, as enunciated by him is as follows: "The enamel is decalcified by acids elaborated by bacterial fermentation of the carbohydrates in the mouth. The bacteria now enter the softened tooth and destroy it by their ferments." This is, in general, the accepted theory of to-day, though it fails to answer a number of important questions. Immunity to caries, localization of caries, sudden halt in the process and eventual repair are questions that remain open and are not accounted for by this theory.

Subsidiary theories have been introduced, but in the main the hypothesis of decay as formulated by Miller holds the arena to-day. Kirk (1914) advanced the hypothesis that bacteria penetrate the enamel and ferment sugar from the blood plasma, thus liberating acid which dissolves enamel from the inside. Baumgartner (1913), from a study of a series of histological preparations, suggested that primary caries is not a chemical but a purely parasitic process. He calls primary caries a streptomycosis. According to him streptococci enter the interprismatic spaces, and disintegrate the interprismatic material, thus destroying the enamel. They then burrow further, and penetrate the dentin and eventually reach the pulp. This appears to be a rather fantastic

explanation and illustrates the facility with which theories may be conceived.

Miller's studies on the bacteriology of caries can, however, be considered only as the great work of a great pioneer. Like other pioneers of science, he accomplished wonderful things with the crude instruments and methods at his disposal. But his work was nevertheless incomplete considered from the point of view of modern bacteriology, and a revision of the subject is not only desirable but highly necessary. Since Miller's work an important contribution has been made by Goadby. But, like Miller, Goadby failed to pay any attention to the mouth anerobes. In 1905 Rodella published an interesting paper in which he claimed that anerobes are the cause of dental caries. His work has not hitherto been confirmed. Recently Kantorowicz published an extensive bacteriological and histological study of caries, in which he confirms in the main Goadby's findings but makes no mention of Rodella's work. The published records of these few observers comprise the important contributions to the subject of the bacteriology of caries.

Neither Miller nor any of the subsequent workers has succeeded in finding an organism specific for caries. All of them report, however, the presence of certain types which they claim are always observed in carious teeth. Miller, Goadby, Sieberth, and Kantorowicz lay stress on the organisms that produce lactic acid, the last three considering the streptococci of the greatest etiological significance. Rodella, as stated above, emphasizes the importance of putrefying anerobic forms like putrificus, butyricus, and the like, which he found in saliva. The unavoidable objection to the work of the former investigators is that they made no attempt to test for anerobes and that streptococci, even if not very abundant, outgrow the other organisms so rapidly that they might appear to be the most important. Rodella's work on the other hand is not carefully controlled and consists merely of an examination of saliva and not of decayed dentin.

To one acquainted with the rich flora constantly present in the mouth it must appear obvious that the types isolated, and hence the types considered of most significance, will depend very largely on the methods employed. Two workers using the same technique will undoubtedly find approximately the same types of

organisms. The exact opposite may be the case when two workers or even the same workers employ different methods. Both results are correct but neither tells more than a part of the truth, and in neither case is there a determination of the relative importance and prevalence of the types reported.

Miller mentions six organisms isolated by Vignal and Gallippi, who examined 18 cases of decayed teeth. The meagerness of the description can be noted from the following summary:

(a) Short, thick bacillus, 1.5 μ long and about as thick; liquefies gelatin in 3 to 4 days; colonies white to opaque.

(b) Long bacillus 3 μ x ½ μ, spreading colonies.

(c) Similar to (b); square ended; does not liquefy gelatin.

(d) Small, thin bacillus, almost like coccus; yellow trail in stab; liquefies gelatin.

(e) Bacillus, rounded ends; liquefies gelatin.

(f) Large coccus.

Such descriptions tell nothing more than that bacilli apparently predominated in the decayed teeth examined by these authors. What types they were is conjectural.

Goadby divides the bacteria of caries into liquefying and non-liquefying groups, and also according to whether they are derived from deep or superficial layers. He finds, strangely enough, the acid bacteria predominant in the deep layers and the liquefiers in the superficial layers.

- Deep layers....
  - Acid formers..... — *Str. brevis*, *B. necrodentalis*, *Staph. albus*
  - Liquefiers..... not isolated.
- Superficial layers
  - Acid formers..... — *Str. brevis*, *St. albus*, *St. aureus*, *S. lutea*, *S. aurantiaca*, *S. alba*
  - Liquefiers..... — *B. mesentericus ruber*, *B. mesentericus vulgatus*, *B. mesentericus fuscus*, *B. mesentericus ferrus*, *B. gingivae pyogenes*, *B. fluorescens liquefac. motilis*, *B. subtilis*, *B. proteus*, *B. plexoformis*

Practically all the liquefiers are members of the hay-bacillus group, which are always present in the mouth as well as everywhere else, and can hardly be claimed to have any special significance.

Dobrzyniecki reports a series of organisms agreeing with some of those found by Goadby. He isolated the following:

B. gangraenae pulpalis (mesentericus?)
St. aureus
Str. pyogenes
S. lutea
St. albus

Kantorowicz, who followed Goadby's technique rather closely, reported results closely agreeing with those of the latter. He finds in the deepest layers streptococci, non-liquefying staphylococci, and two types of bacilli—one very similar to Goadby's *B. necrodentalis*. He concludes that in the deepest layers liquefying forms are not present. The streptococci are considered by him to be the main cause of caries.

Rodella, using a different technique, claims that *B. putrificus* and *B. phlegmones* are always present in carious teeth. Both of these are strictly anerobic organisms of putrefaction. His view is that the butyric acid-producing anerobes initiate and complete decay of teeth.

Which of these authors is right and what actually is the flora of carious teeth? The role of *oxygen* in regulating the growth of these organisms may afford a clue to the answer. There is progressively less oxygen in a carious cavity, from the superficial layer of the decayed mass inward. It may be impossible for aerobic liquefiers to thrive in the deepest portion of the carious mass, with consequent diminution in the number of these types, but *anerobic* and facultative anerobic cells may alone be active in the " deep layer," thus accounting for the contradictory findings in this relation. Anerobic forms of importance may have failed of detection in carious teeth, in the work of our predecessors, because of their failure to impose suitable conditions of cultivation. A new aspect of the influences operative in dental caries may be presented from this standpoint.

We shall return to this phase of the subject in a succeeding section of this report.

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* The most important contributions are indicated by asterisks.

CHEMICAL STUDIES OF THE RELATIONS OF ORAL MICROÖRGANISMS TO DENTAL CARIES

By
WILLIAM J. GIES AND
COLLABORATORS

3. A BIOCHEMICAL STUDY AND DIFFERENTIATION OF ORAL BACTERIA, WITH SPECIAL REFERENCE TO DENTAL CARIES (Continued). (II)

By I. J. KLIGLER

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CHEMICAL STUDIES OF THE RELATIONS OF ORAL MICROÖRGANISMS TO DENTAL CARIES[1]

BY WILLIAM J. GIES AND COLLABORATORS.

3. A Biochemical Study and Differentiation of Oral Bacteria with Special Reference to Dental Caries (continued) (II)[2]

BY I. J. KLIGLER.

(*From the Biochemical Laboratory of Columbia University, at the College of Physicians and Surgeons, New York.*)

II. EXPERIMENTAL.

(First section)[3]

1. **Plan of Work.**—From a general survey of the literature, and from a few preliminary experiments, it became evident that methods other than those utilized in the past would be necessary in order to avoid most of the errors of earlier workers. The variety of oral bacteria, as was pointed out before, is legion. The types of real significance, on the other hand, are relatively few in number. The accidentally occurring strains are presumably few in number, whereas those of real significance are relatively numerous. If common qualitative culture methods are employed with ordinary media, however, relatively unimportant individuals overgrow the less cultivable but more important organisms and assume a prominence that is devoid of oral significance.

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[1] Reports of findings in investigations conducted under the auspices of the First District Dental Society of the State of New York.

It had been our intention to present, in this issue of the *Journal*, the remainder of the report on our work for the academic year 1913-1914, as already suggested by us (*Journal of the Allied Dental Societies*, 1915, x, p. 137), but details in our recent bacterio-chemical study have become so numerous that publication, in this issue, of the preliminary *experimental* data in our report for 1914-1915 is particularly desirable, as a further prelude to our oral report, next November, on the *chemical* findings. The concluding portion of the report for 1913-1914, on effects of food-acid media, will be published in the next issue of this *Journal* with the remainder of the report for 1914-1915.

The introductory and historical portions of these studies were published in the last previous issue of the *Journal of the Allied Dental Societies* (pp. 137 and 141).

[2] Accepted by the executive officer of the Department of Biological Chemistry of Columbia University as *Part II* of a dissertation, submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, in the Faculty of Pure Science, Columbia University, June, 1915.

The bacteriological experiments were conducted in the laboratory of the Department of Public Health of the American Museum of Natural History, under the supervision of the executive officer of the Biochemical Department of Columbia University. At Dr. Gies's request we were privileged to obtain, at the Clinic of the New York College of Dental and Oral Surgery, numerous oral specimens. We are indebted to Drs. Louise C. Ball and Frank L. Chambers for courteous and very helpful coöperation in this connection.

[3] The concluding section of this dissertation will be published in the succeeding issue of the *Journal of the Allied Dental Societies*.

The same observation was made by MacNeal (21) and his co-workers in their admirable study of the fecal flora of man.

As a result of these considerations it was decided to adopt a quantitative technique and to employ media suitable for the purpose. Serious difficulties were encountered, however, in determining the methods to be used and the kind of media best adapted for the oral flora. In the first place, it was clear that an examination of saliva alone would not serve our purpose, for a study of the flora of saliva would reveal relatively little regarding the nature of the flora in the deposits on teeth. It was an essential part of the plan to make a systematic study of the bacteria in ordinary dental deposits and in decayed enamel, dentin and pulp; but the amount of deposit on normal clean teeth is usually very small, and the collection and weighing of such minute quantities of material, under the required conditions of sterility, offered serious difficulties. So also, it was hard to decide what media to use. Glucose-agar seemed desirable but, on that mixture, the streptococci and acid-forming diplococci grew so rapidly and elaborated so large an amount of acid that the slowly growing types never got a chance to make a start. Gelatin might have been useful were it not for the fact that most of the typical mouth flora fail to grow at room-temperature. After a considerable amount of preliminary work the following series of methods was adopted.

2. **Methods.**—COLLECTION AND WEIGHING OF SPECIMENS. A small glass vial, about ¾ cm. in diameter and 4 cm. long, and weighing about 4 gm., was tightly closed with a one-hole rubber-stopper in which was fitted a narrow glass-rod drawn out in delicate spatula-form at the inserted end. (See Figure, page 284.) The tip could easily be broken from this delicate spatula. The vial was covered, at the upper part, with tin-foil and sterilized in an autoclave under 20 lb. pressure for 5-10 minutes. The vial was then placed upright in a desiccator until ready for use. Just before using the vial, the tin-foil was carefully removed and the vial weighed accurately to milligrams. The spatula was then cautiously withdrawn by means of the rubber stopper, the vial being held with a sterile dry cloth; deposit on a tooth or teeth was carefully removed on the spatula which was then returned to the tube, the tin-foil replaced and the whole put in a desiccator until ready for sampling. The time between the collection

of the specimen, and the weighing and plating, was never more than an hour, usually less; often, as in the case of the specimens from healthy teeth, only five or ten minutes elapsed. Just before plating, the vial, with the scrapings, was again weighed in order to determine by difference the weight of the material obtained. The spatula was then carefully removed and inserted into the mouth of a sterile dilution bottle. By slight pressure of the rod against the inside of the bottle the spatula-tip was broken off at the drawn-out part and fell into the dilution bottle.

SAMPLING OF SPECIMENS. The bottle contained 10 c.c. of sterile water and a number of sterile round glass beads. After the specimen had been added the bottle was shaken vigorously to produce a uniform suspension. This was used as a stock supply; and, by withdrawing volumes of 1 c.c. and mixing them with various amounts of sterile water, any number of desirable dilutions could be prepared. The dilutions were made with one-tenth of the original material in a definite quantity of water; corrections were made later on the basis of actual mass of substance present.

DETERMINATIONS OF THE NUMBER OF BACTERIA. *Microscope-Count.* That cultural methods reveal only a small fraction of a bacterial flora is well known. Winslow (32) found that the microscope-count in sewage effluents is often one hundred times as great as the plate-count. MacNeal, Latzer and Kerr (21), in their study of fecal flora, noted even a greater divergence

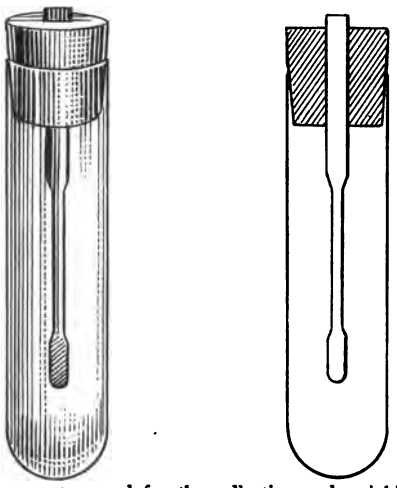


Diagram of vial-apparatus used for the collection and weighing of specimens.

between the two counts. Several causes probably contribute to this difference. There are present, very likely, many bacteria that are not cultivable under given artificial conditions, or are kept out by their more greedy, more readily adaptable or hardier associates. The discrepancies may also be accounted for, in part, by the extreme difficulty in breaking up bacterial clumps.

An interesting question arises in this connection. Are the many uncultivable bacteria living cells that would continue to develop in their natural environment but are unable to do so in the artificial environment? Klein (16) claims that the bacteria (fecal) which fail to develop are dead. Winslow, on the other hand, found that when pure cultures of a cultivable bacillus were used, the plate- and microscope-counts were the same, and that dead cells rapidly disintegrated and failed to stain with the anilin dyes. It is quite likely, therefore, that most of the stained cells seen in a microscopic field represent living organisms, capable of developing under favorable conditions. Hence a microscope-count is of considerable value as indicating the total flora present under given conditions.

A number of methods for direct counts have been suggested at various times. The one devised by Winslow (32) for his sewage work was found satisfactory for our purpose; and, except a few determinations with the method used in counting milk, was employed in all the experiments. The method, to quote Winslow (32), "consists in spreading a definite amount of the emulsion evenly on a cover-slip and allowing it to dry in the air." After drying, it is fixed by passing through the flame, covered with Ziehl-Nielsen's carbol-fuchsin solution, warmed till steam rises, washed, dried and mounted. In my work 0.1 c.c. of the original 10 c.c. of stock solution was spread uniformly over a square cover-slip ¾ inch on each side, and allowed to dry at room temperature. The preparation was then fixed in a flame, stained with steaming fuchsin, mounted on a slide so that the diagonal of the slip was parallel with the long edge of the slide, and squares counted with the Sedgwick-Rafter micrometer eye-piece. Usually 20 fields were counted in different parts of the cover-slip and the average taken. The microscopic field was so adjusted that a factor of 50,000 was obtained (the area of the field times the area of the cover-slip), which,

when multiplied by the count, gave the number of bacteria in 0.1 c.c. or 0.01 of the total mass used. Multiplying this figure by 100 and dividing by the number of milligrams in the original mass, the total number of bacteria in 1 mgm. of oral material was obtained.

In general it was found that under normal conditions, the number of bacteria varied directly with the mass of the deposit on the teeth; whereas under pathological conditions, the count varied with the stages of decay.

Plate-Method. For quantitative determinations, four different sets of plates were made: (a) Litmus-glucose-agar and (b) agar plates, both of which were incubated at 37° C., under aerobic conditions. (c) Litmus-glucose-serum-agar plates, incubated at 37° C. in an anerobic jar, using the combination of exhaustion and alkali pyrogallol. This method did not always give satisfactory anerobic conditions. (d) Glucose-agar plates were poured and rendered anerobic according to the method suggested by Krumwiede (19). This method gave satisfactory anerobiosis but introduced another serious difficulty in the large number of spreaders.

The four sets of plates presented different conditions and there was considerable variation in the flora on each set. Gelatin plates were not made since preliminary tests of, and plates from, the first few samples showed that most mouth forms fail to develop at room temperature. All the plates were generally incubated for from 4-5 days, this long period being especially important in bringing to light the slow growing leptothrix type.

Different dilutions were made in the usual manner from the original stock suspension, and plates made from the higher dilutions only. The dilutions used for plating depended, of course, on the mass of the scrapings and on the abundance of bacteria in it. After the incubation period the plates were counted, the average from the various plates taken and the number of bacteria per milligram of the original mass, under each condition, calculated.

QUALITATIVE STUDY OF TYPES. Of the different sets of plates those showing least crowding were examined microscopically with low magnification, and characteristic colonies were transferred to an appropriate medium (agar, glucose-agar or serum-

glucose-agar) and retained for more detailed biochemical and morphological study. Attempts were made to obtain representative colonies so as to get an approximate idea of the relative importance of the various types. Since all the colonies were picked from dilutions of 1 : 1000 or over, the selected forms represented only those types of organisms which were relatively most abundant in a particular specimen. It will be noted later that the prevailing types of organisms varied with the condition of the teeth—one type of flora displacing others in a definite and uniform manner.

To make sure that the anerobes which Rodella claimed to have found in saliva would not escape detection, if any were present, tubes of the meat-egg mixture recommended by Rettger (27) were incubated under anerobic conditions at 37° C. for 3-4 weeks. Anerobic plates and deep glucose-agar shake-cultures were then made and typical colonies fished and examined. This method was very useful for the isolation of anerobes that were often overgrown and hence not detected on the direct plates.

3. **Sources of Material.**—Specimens were taken from forty individuals ranging from "immunes" (people who never had caries or only slight defects) to those whose teeth were in the last stages of decay. Twenty of the specimens were taken from healthy teeth in mouths of various degrees of cleanliness with the object of obtaining as broad a survey of the prevailing conditions in ordinary "clean" and "dirty" mouths as was possible in the time available. This survey was also intended to serve as a basis for comparisons of the flora obtained from the pathological cases. The other twenty samples were taken from decayed teeth in different stages of decay. This material was obtained either with the glass spatula described above (Fig. 1) or with the aid of a sterile excavator. The patients were examined by trained dentists and the exact diagnoses made by them were recorded. Specimens were thus obtained from teeth in the primary stages of decay as well as from teeth in which the dentin and pulp also were affected.

Among the first twenty specimens are some taken from the same individual on different occasions either before the morning brushing and after, or before and after the noon meal. In this

manner it was hoped to obtain a general preliminary indication of effects of the environment (the condition of the mouth, food, etc.) on the character of the flora. It would have been desirable thoroughly to study these phases of the problem; it was thought best, however, for a survey of this sort, to select as many distinct types as could be conveniently handled without incurring risk of the confusions that might result from premature extension of our studies at this stage of their progress.

4. **Results.**—BACTERIOLOGICAL DATA. The cases studied fall into two main divisions—normal and carious teeth, respectively, each of which groups may again be subdivided into subgroups based either on the condition of cleanliness of the mouth or on the extent of the decay. The division between "clean" and "dirty" mouths was, of course, somewhat arbitrary; but, in the case of the carious teeth, the separation was based on the diagnosis of trained dentists. These groups are summarized below; the various results obtained will be presented under these heads.

A. *Normal Teeth.*—

(1) Material taken from healthy teeth of "immune" individuals.

(2) Material taken from healthy teeth of individuals whose mouths were cleaned regularly but who at one time or another have had caries.

(3) The same as (2) but from individuals whose mouths were "dirty" and poorly cared for.

(4) Material taken from healthy teeth, from mouths in various conditions of cleanliness, in the morning before the usual brushing and after.

(5) Same as (4) but taken before and soon after the noon meal.

B. *Carious Teeth.*—

(6) Material taken from teeth in the first stages of caries, the enamel only being affected.

(7) Material taken from teeth in which both enamel and dentin were involved.

(8) Material taken from teeth in which decay had gone far enough to expose the pulp, though the latter was not involved.

(9) Material taken from teeth in the final stages of decay, the pulp being involved.

QUANTITATIVE RESULTS: NORMAL TEETH. *Group 1.* The quantitative results include both the plate- and microscope-counts. As was to be expected, the microscope-count was considerably higher, even if the aerobic and anerobic plate-counts are totaled as distinct values. Unfortunately, " immunes " are not so easy to obtain, and of the twenty samples from normal teeth only two were taken from individuals who have never had caries. In each case the mouth was carefully cleaned regularly and the dentist visited about once a year. The amount of dental deposit was small, and both the microscope- and plate-counts were relatively low. Roughly, the microscope-count was 5-6 times as great as the combined aerobic and anerobic counts on glucose-agar. The results for this group are indicated in Table I.

From the chemical and biological standpoints it is interesting to find that the number of organisms that develop on the glucose-containing medium is greater even in this group than that on the ordinary agar. The *normal* oral flora is, on the whole, distinctly *acid-producing* in character. It is also notable that a larger number of organisms seems to develop under anerobic conditions on the glucose-containing medium.

TABLE I.—DATA PERTAINING TO TEETH "IMMUNE" TO DENTAL CARIES.

No. of specimen	Source of specimen	Condition of mouth and teeth	Microscope-count No. per mg.	Plate-count. No. per mg.			
				Aerobic		Anerobic	
				Agar	Gl.-agar	Krumwiede	Jar
3	Deposit on incisors and canine.	Very clean. Teeth in fine condition.	15,000,000	350,000	430,000	2,000,000	.....
9	Deposit on incisors and bicuspids.	Very clean. Teeth in fine condition.	6,000,000	200,000	680,000	640,000	300,000

Group 2. The cases falling into the second group show on the whole (as seen from Table II) a higher microscope-count, though the plate-count is not materially increased. The striking point for this group is the high ratio of the microscope- to the plate-count, ranging from 12 to 1, to 40 to 1, and averaging 13 to 1. The average count for this group is generally twice that of Group 1. (See Table X). Another suggestive fact is the apparent, though less marked, increase in the number of cultivable bacteria for the last four cases in Table II. These subjects, while giving as much attention to their teeth as those referred to in the first part of the table, were nevertheless much less successful in preventing dental deterioration. There may be a definite relation between the number of bacteria capable of developing on teeth and susceptibility to decay. Here again the presence of sugar in the culture medium is on the whole distinctly favorable for the development of these types.

Group 3. The data in Table III indicate two things: First, an enormous increase in both the microscope- and plate-counts

TABLE II.—DATA PERTAINING TO DEPOSITS ON HEALTHY TEETH IN "CLEAN" MOUTHS.

No. of specimen	Source of specimen	Condition of mouth and teeth	Microscope-count No. per mg.	Plate-count. No. per mg.			
				Aerobic		Anerobic	
				Agar	Gl.-agar	Krumwiede	Jar
2	Deposit on incisors and canine.	Mouth clean; examined regularly. Had caries before.	.....	160,000	210,000	2,000,000	.....
11	Deposit on canine and bicuspids.	Mouth and teeth cleaned regularly. Teeth in good condition.	25,000,000	350,000	190,000	500,000	450,000
13	Deposit on bicuspids and first molar.	Teeth in good condition now. Mouth cleaned regularly.	16,500,000	133,000	155,000	150,000	255,000

TABLE II (*con.*).—DATA PERTAINING TO DEPOSITS ON HEALTHY TEETH IN "CLEAN" MOUTHS.

No. of specimen	Source of specimen	Condition of mouth and teeth	Microscope-count No. per mg.	Plate-count. No. per mg.			
				Aerobic		Anerobic	
				Agar	Gl.-agar	Krumwiede	Jar
12	Deposit on bicuspids and molars.	Mouth cleaned regularly. Teeth in good condition.	40,000,000	160,000	200,000	140,000	140,000
1	Deposit on incisors	Mouth cleaned regularly. Teeth in good condition; recently treated for caries of lower left molar.	.....	2,300,000	6,000,000	2,000,000	....
5	Upper incisors and canine	Teeth in poor condition; many caps and fillings. Mouth cleaned regularly.	17,500,000	750,000	500,000	.....	750,000
14	Deposit on healthy bicuspids.	Mouth cleaned regularly. Teeth in poor condition.	25,000,000	550,000	550,000	520,000	570,000
15	Deposit on incisor adjacent to carious canine.	Mouth cleaned regularly. Teeth in poor condition.	23,000,000	80,000	100,000	530,000	440,000

for "dirty" mouths; second, marked effect of tobacco on the number of bacteria in "dirty" mouths. For the individuals subject to the habit of chewing tobacco the microscope-count is the same as for those indicated in Table II, whereas the plate-count

is appreciably lower. Tobacco apparently serves the same purpose as the tooth-brush. Despite the increase in total numbers, the ratio for this group is about the same as for Group 2.

TABLE III.—DATA PERTAINING TO NEGLECTED TEETH IN "DIRTY" MOUTHS.

No. of specimen	Source of specimen	Condition of mouth and teeth	Microscope-count No. per mg.	Plate-count. No. per mg.			
				Aerobic		Anerobic	
				Agar	Gl.-agar	Krumwiede	Jar
10	Deposit on incisors.	Mouth "dirty." Thick deposit on teeth. Cleaned irregularly. "Does not suffer from tooth trouble."	54,000,000	1,090,000	1,800,000	1,100,000	1,000,000
4	Deposit on bicuspids.	Mouth "dirty." Thick deposit on teeth. Rarely cleaned. Has never been to a dentist.	41,000,000	1,670,000	1,920,000	3,000,000	.....
7	Scrapings from incisors.	Mouth in poor condition. Teeth cleaned infrequently. Chews tobacco. Teeth browned.	.....	160,000	500,000	400,000	80,000
6	Scrapings from healthy incisors and bicuspids.	Mouth "dirty." Rarely cleaned. Chews tobacco. Teeth brown. Many teeth missing.	25,000,000	330,000	170,000	250,000	200,000

Groups 4 and 5. The comparison between the counts ob-

tained before and after brushing, and before and after the noon meal, are shown in Tables IV and V respectively. Though the specimens were not taken on the same day before and after, and despite the fact that only three specimens were taken in each series, the results are so uniform and striking that there can be no doubt of their general correctness. The first series indicates that brushing removed at least ¾ of the total number of bacteria on the teeth. The second series indicates that the noon meal had the effect of doubling the total number of bacteria on the teeth. In the specimens taken before brushing and after the meal, the number of cultivable bacteria was higher than in those taken after the brushing and before the meal, respectively. The number of colonies on the agar-plates was just as large as that on the sugar-containing medium. The ratio between microscope- and plate-counts is as low as 4:1 and 7:1, respectively.

These results appear to be the first quantitative counts of a comparative character that have ever been made on bacteria of the teeth. In this connection we note the role that might be played by the small quantities of tooth-scrapings and food-rests introduced into the medium and the stimulating effect they might have on the growth of these organisms. This, with many other suggestions from these findings, will receive due attention as these studies develop.

QUANTITATIVE RESULTS: CARIOUS TEETH. The specimens taken directly from carious teeth cannot be readily divided into groups, as the affections insensibly grade into one another. Four divisions have been made as outlined above, but the demarcation is not sharp. "Overlapping" conditions are represented by four of the specimens: two that might be classed with Group 7 and two with Group 9. In these cases the pulp is only slightly involved or not at all, while the bacteriological picture consists of a composite of the types of organisms in both the second and last stages of decay. In the discussion of the frequency and abundance of types, these four specimens are classed together as Group 8.

TABLE IV.—DATA PERTAINING TO TEETH IN THE SAME INDIVIDUALS BEFORE AND AFTER THE USUAL MORNING BRUSHINGS.

No. of Specimen	Time when specimen was taken	Individual from whom specimen was taken	Microscope-count No. per mg.	Plate-count. No. per mg.			
				Aerobic		Anerobic	
				Agar	Gl.-agar	Krumwiede	Jar
3	Before brushing	K.	65,000,000	10,000,000	7,500,000	8,000,000	.....
17	After brushing	K.	15,000,000	350,000	430,000	2,000,000	.....
16	Before brushing	Kr.	70,000,000	4,200,000	2,300,000	1,700,000	1,030,000
5	After brushing	Kr.	17,500,000	750,000	500,000	.....	750,000
18	Before brushing	W.	70,000,000	18,500,000	12,500,000	11,400,000	8,000,000
1	After brushing	W.	.....	2,300,000	6,000,000	2,000,000	.....

TABLE V.—DATA PERTAINING TO TEETH IN THE SAME INDIVIDUALS BEFORE AND AFTER NOON MEALS.

No. of specimne	Time when specimen was taken	Individual from whom specimen was taken	Microscope-count No. per mg.	Plate-count. No. per mg.			
				Aerobic		Anerobic	
				Agar	Gl.-agar	Krumwiede	Jar
12	Before the meal	H.	40,000,000	160,000	200,000	140,000	140,000
21	After the meal	H.	100,000,000	7,000,000	4,500,000	6,000,000	8,300,000
13	Before the meal	B.	16,500,000	133,000	155,000	55,000	255,000
20	After the meal	B.	37,500,000	2,500,000	2,650,000	2,500,000	3,000,000
15	Before the meal	P.	23,000,000	80,000	100,000	530,000	440,000
19	After the meal	P.	65,000,000	8,000,000	1,000,000	800,000	1,200,000

Group 6. In this group there were three cases, in each of which the enamel only was affected. The dental deposit was very slight and the amount of scrapings in each instance was only 0.5 mgm. The striking results here are the abnormally high microscope-count, between 450,000,000 and 600,000,000 per mgm. and the comparatively high plate-count. It is noteworthy

TABLE VI.—DATA PERTAINING TO CARIES OF ENAMEL ONLY. PRIMARY STAGE OF DECAY.

No. of Specimen	Source of specimen	Condition of tooth, mouth, gums, etc.	Microscope-count No. per mg.	Plate-count. No. per mg.			
				Aerobic		Anerobic	
				Agar	Gl.-agar	Krumwiede	Jar
32	Upper left incisor.	Layer between dentin and enamel sensitive. Tooth had been filled; decay continued.	630,000,000	1,750,000	2,000,000	4,000,000	.....
30	.....	Break of about one mm. in enamel. Teeth in good condition. Gums healthy.	500,000,000	2,000,000	4,300,000	16,800,000	44,000,000
28	.....	Enamel only affected. Teeth and gums in poor condition. Abscess about adjacent tooth.	460,000,000	*20,400,000	21,200,000	14,000,000	60,000,000

* The enormous increase in this count is undoubtedly due to the abscess in the adjacent tooth.

that the ratio here rises to 25:1, a figure far higher than the average for the normal samples, and that the plate-count for the semi-anerobic conditions obtainable in the jar is appreciably higher than for any of the other plates. Another interesting feature that distinguishes this group, as well as Group 7, from the normal teeth is the occurrence in great abundance of certain types of bacteria that were present only rarely or not at all on the normal teeth. These types will be discussed, at length, later. The detailed results are tabulated in Table VI.

TABLE VII.—DATA PERTAINING TO CARIES OF ENAMEL AND DENTIN. SECOND STAGE OF DECAY.

No. of specimen	Source of specimen; condition of tooth	Condition of mouth, gums, etc.	Microscope-count No. per mg.	Plate-count. No. per mg.			
				Aerobic		Anerobic	
				Agar	Gl.-agar	Krum-wiede	Jar
38	Second lower bicuspid. Proximal cavity involving dentin.	.....	.....	270,000	1,070,000	730,000	130,000
39	Upper left second bicuspid.	Mouth poorly cared for.	93,500,000	2,700,000	2,300,000	1,900,000	1,250,000
40	First lower right molar; enamel and dentin involved.	Mouth and teeth in poor condition.	142,000,000	600,000	1,200,000	600,000	280,000
31	Upper right incisor. Enamel and dentin involved; dentin much decayed.	Gums healthy. Many similar cavities in teeth.	500,000,000	1,900,000	1,500,000	1,800,000	2,200,000
29	Upper right incisor. Enamel and dentin affected; pulp not involved.	Much calculus and many erosions. Teeth neglected.	300,000,000	1,450,000	9,600,000	3,700,000	22,000,000

Group 7. This group (Table VII) consists of five cases of caries in which both the enamel and dentin were involved. On the whole, this group is characterized by relatively larger amounts of deposit, mostly debris, food particles, etc., and lower microscope- and plate-counts than Group 6. This is probably due to several factors, among which the accumulation of waste is perhaps of some significance in suppressing the growth of the bacteria. The large amount of debris undoubtedly serves, also, as a sort of diluent for the bacteria, which *in toto* equal those for the first stage of caries, where the scrapings consisted almost entirely of bacteria. That this is the case is seen when one multiplies the figures for the total weight of the material obtained, by those for the count per mgm. This idea is also confirmed by the results obtained for the specimens mentioned in Table IX, which consisted almost entirely of debris and food particles. Otherwise there is no marked difference between these cases and those in which the enamel only was involved. The prevalent types of organisms were essentially the same in both groups except in two cases, which apparently represented the transitional types mentioned above. (Group 8.)

Group 9. This group includes all cases in which decay has progressed sufficiently to involve the pulp. Altogether there were 9 samples, 2 of which form a sort of transitional class showing some of the characteristics of the previous groups and some of this one. (Group 8). These two gave the high microscope- and plate-counts, the relatively higher counts on the glucose-agar and on the serum-agar plates kept under anerobic conditions, and types of organisms associated with the decay of enamel and dentin as well as of the pulp. These respective types are, furthermore, less abundant in these cases than for either of the other phases of decay.

Group 9 as a whole is characterized by a moderately high microscope-count and a relatively higher count on the anerobic plates generally, associated with gas production and a putrid odor. (See Table VIII). Further distinguishing characteristics of this group, which show that it is entirely distinct from those related to the primary stages of tooth-decay, are the occurrence in great abundance in all cases of anerobic putrefying organ-

isms, and the practically complete absence of certain types that are distinctive of decay of enamel and dentin. These organisms, their abundance and frequency of occurrence, will be discussed more fully later; attention is called here merely to the correlation between the clinical picture, the total counts and the specific types of organisms found.

TABLE VIII.—DATA PERTAINING TO PUTRESCENT PULP.

THIRD AND FOURTH STAGES OF DECAY.

No. of specimen	Source of specimen	Condition of tooth, mouth, gums, etc.	Microscope-count No. per mg.	Plate-count. No. per mg.			
				Aerobic		Anerobic	
				Agar	Gl.-agar	Krumwiede	Jar
8	First lower left molar, pulp putrescent.	Mouth "clean." Teeth in good condition. Tooth affected was filled about two months ago.	80,000,000	880,000	1,740,000	1,700,000	1,120,000
22	Lower left third molar, pulp putrescent.	Much calculus. Gingivitis. Pus at gum line.	36,500,000	6,350,000	1,810,000	2,000,000	2,000,000
23	Lower incisor, putrescent pulp.	Pyorrhoea; much recession of gums.	41,000,000	240,000	1,430,000	5,700,000	.....
24	First upper right molar. Deep cavity, putrescent pulp.	.....	23,500,000	1,500,000	360,000	1,550,000	900,000
25	Upper left incisor. Pulp putrescent.	Pericementitis.	85,000,000	155,000	125,000	.....	.....

TABLE VIII (*con.*).—DATA PERTAINING TO PUTRESCENT PULP. THIRD AND FOURTH STAGES OF DECAY.

No. of specimen	Source of specimen	Condition of tooth, mouth, gums, etc.	Microscope-count No. per mg.	Plate-count. No. per mg.			
				Aerobic		Anerobic	
				Agar	Gl.-agar	Krumwiede	Jar
26	Last left molar. Pulp partly decayed.	Gingival cavity.	110,000,000	150,000	500,000	24,000,000	260,000
36	First lower right molar. Pulp chamber exposed; putrescent.	Teeth in bad condition. Other cavities in mouth.	40,000,000	60,000	250,000	1,000,000	750,000
27	Upper right incisor. Dentin much decayed; pulp involved.	Marked erosion and calculus.	61,500,000	1,600,000	2,800,000	2,400,000	11,000,000
34	First lower left molar. Third stage.	Gums healthy. Much bridge-work. Mouth "clean."	41,000,000	500,000	500,000	1,750,000	.....

Still another sub-group of three samples is included here, representing the material deposited on the surface and in the upper part of the cavity of putrescent caries, which consists of a considerable amount of debris, food rests, etc. The microscope-count for these specimens is comparatively low, the plate-counts relatively high, and the amount of accumulated deposit much greater than usual. The types of bacteria for this sub-group are also characteristic, consisting almost entirely of cocci. The data are shown in Table IX.

QUALITATIVE RESULTS. It was a comparatively simple matter, once the method of sampling was defined, to pour plates and

TABLE IX.—DATA PERTAINING TO MATERIAL TAKEN FROM SUPERFICIAL LAYER IN DECAY OF EXPOSED PULP.

No. of specimen	Source of specimen	Condition of mouth	Microscope-count No. per mg.	Plate-count. No. per mg.			
				Aerobic		Anerobic	
				Agar	Gl.-agar	Krumwiede	Jar
33	Second bicuspid. Pulp chamber affected down to root-canal. Material taken from top.	Much calculus and stain. Tooth pyorrheal.	16,000,000	2,240,000	4,160,000	2,560,000	.....
35	Temporary molar; fourth stage. Pulp exposed.	Mouth fairly clean. Gums healthy.	70,000,000	1,800,000	7,300,000	10,200,000	.....
37	Upper layer of material on first molar. Pulp putrescent; exposed.	Teeth in bad condition. Other cavities in mouth.	25,600,000	410,000	240,000	500,000	250,000

make counts. The important part of the problem was, however, to determine the types of bacteria found on normal and decayed teeth, respectively. This was not quite so simple, because of the great complexity and bewildering pleomorphic character of the oral flora. The first difficulty was greatly reduced by selecting only those types which were sufficiently abundant to appear on plates containing a dilution of 1:1000 or over. The second problem was solved by subjecting isolated types to somewhat thorough biochemical tests.

The inadequacy of purely morphological grouping is nowhere more strikingly illustrated than in the case of some of the bacteria found in the mouth. Bacteria as a class have evolved so little along morphological lines that it is impossible to differentiate members of the same genus on a merely physical basis. Bacteriologists, therefore, resort to the more delicate criteria of protoplasmic constitution and physiological activity, in which directions remarkable differentiations exist. Bacteria generally

have evolved in certain main directions: thus, one group has acquired marked carbohydrate-splitting properties, another has developed the property of digesting various protein substances, while still another possesses both powers. The streptococci belong to the division showing but little tendency to proteolysis, the aerobic spore-formers belong to the proteolytic group, while the *B. proteus* and the anerobic spore-formers are capable of digesting both carbohydrates and proteins.

In a study of this nature it is necessary, therefore, to ascertain the carbohydrate and nitrogenous metabolism of the various types. The former is ordinarily determined by observing gas and acid production in media containing various carbohydrates, the latter by testing for indol and ammonia, end-products of nitrogenous metabolism, in a sugar-free peptone-mixture. The proteolytic activities of bacteria may be further studied by observing their action on gelatin, casein or serum, the presence or absence of protease being indicated by presence or absence of liquefaction. A further index of the metabolic activities of bacteria is their reducing action on stable compounds such as sodium or potassium nitrate. These tests help to differentiate the various types of bacteria into comparatively well defined groups.

Since the spirochetes and fusiform bacilli have been studied rather extensively, and also necessitate a very special and particularly difficult technique for their isolation, it was decided to disregard these organisms temporarily and devote our chief attention to other forms that have not been investigated so thoroughly. So much confusion exists regarding the so-called *Leptothriceæ,* and the anerobic flora, that it was desirable to pay special attention to these forms. It is only by clearing up obscure points that one can hope to throw new light on any subject. This plan was not adopted, however, to the entire exclusion of other significant types. An attempt was made to include practically all the prevailing cultivable types as they occurred on the plates under examination. To this end proportionate numbers of characteristic colonies were picked from each set of plates. Those taken from the aerobic plates were streaked on glucose-agar while those from the anerobic plates were inoculated in glucose-agar stabs. These cultures were then tested on various culture media.

TABLE X.—AVERAGE VALUES IN THE COUNTS FOR THE DIFFERENT GROUPS (SEE CHARTS A AND B) OF TEETH REFERRED TO IN TABLES I-IX.

Group	No. of cases in each group	Description of Group	Microscope-count. Average No. per mgm.	Plate-count. No. per mgm.				Ratio * between microscope- and plate-counts
				Aerobic		Anerobic		
				Agar	Glucose-agar	Krumwiede	Jar	
1	2	Immune cases	10,500,000	275,000	550,000	1,320,000	300,000	6:1
2	8	Healthy teeth; "clean" mouths	24,500,000	650,000	990,000	835,000	435,000	13:1
3	2	Healthy teeth; "dirty" mouths	47,000,000	1,380,000	1,860,000	2,050,000	1,000,000	12:1
(3,a)	2	Healthy teeth; "dirty" mouths; tobacco chewers	25,000,000	245,000	335,000	325,000	140,000	38:1
4	3	Healthy teeth, before and after brushing	68,000,000 16,250,000	10,900,000 1,700,000	7,400,000 2,300,000	7,370,000 2,000,000	4,520,000 750,000	4:1 4:1
5	3	Healthy teeth, before and after meal	26,500,000 67,500,000	124,000 5,830,000	152,000 2,720,000	342,000 3,100,000	278,000 4,160,000	60:1 7;1
6	3	Primary caries	530,000,000	8,050,000	9,200,000	11,600,000	54,000,000	25:1†
7	5	Caries; enamel and dentin	258,900,000	1,380,000	3,130,000	1,750,000	5,170,000	31:1
(9,a)	3	Pulp exposed; superficial layer	37,200,000	1,480,000	3,900,000	4,420,000	.....	45:1
9	9	Pulp putrescent, deep layer. 3rd and 4th stage of decay	57,670,000	1,270,000	1,060,000	5,010,000	2,670,000	9:1

* In obtaining the ratio the plate-count used is the sum of the aerobic and anerobic counts on the glucose-agar plates.

† If the jar-count were used the ratio would be 9:1, but this jar-count seems aberrant and the Krumwiede plate-count is used, as in all the other cases.

THE LENGTH OF EACH VERTICAL BAR CORRESPONDS TO THE NUMBER OF BACTERIA PER MILLI-
GRAM OF DENTAL DEPOSIT, AS ASCERTAINED FOR A SINGLE SPECIMEN

The tests to which the cultures were subjected were the following:

A. Morphology and chromology (Gram-stain)
B. Motility
C. Spore-formation
D. Aerobiosis
E. Fermentation of carbohydrates
F. Action on milk
G. Liquefaction of gelatin
H. Action on peptone:
   a. Indol production
   b. Ammonia production
I. Reduction of nitrates

A. *Morphological* examinations of the various organisms were made by staining with gentian violet and by Gram's method. The latter test separated the different strains into two main divisions: those that retained the dye (Gram-positive types) and those that did not retain it (Gram-negative types). These tests also divided the bacteria into large families and genera, *e.g., cocci:* streptococci, diplococci, and staphylococci; *bacilli:* large and small rods, spore-bearing or non-spore-bearing; *trichomycetes:* branched or non-branched threads or short, pleomorphic and long non-pleomorphic threads. More than such a broad sub-division cannot be obtained on a purely morphological basis, with a flora so highly variable as that of the mouth. Some of the types of the trichomycetes are so puzzling that under certain conditions they would readily pass as cocci, under others as diphtheroids and under still others as threads. It is no wonder that Vincentini at one time claimed that these organisms were the progenitors of all the other members of the oral flora. There can be no doubt that they contribute greatly to the marked variability of the microscopic appearance of that flora. Another type, a bacillus, varies in morphology from a short, thin rod of about 2 to 3 μ in length, to long rods and very long threads. Some of these were replated several times to determine whether they were pure cultures.

With these facts on the pleomorphic character of oral bacteria in mind, we can readily see how little reliance may be placed

on studies based solely on morphological examinations; and how much significance may be attached to named species seen only in pictures. Schaudin laid down the rule that the only proper criterion for the classification of protozoa is a determination of their life cycles. A good precept for the study of bacteria would be: The only criterion for the differentiation of bacterial types is a determination of their *biochemical properties*. Only such forms can be spoken of with definiteness.

B. *Motility* was determined in the usual way, by direct microscopic observation.

C. *Spore-formation* was determined by staining and, where doubtful, by the heating test. The culture to be tested was heated, to 80° C. for 10 minutes. If, on sub-culturing, growth was obtained the organism was considered capable of forming spores.

D. *Aerobiosis*. A simple method for determining aerobiosis is to make a stab in a deep tube of glucose-agar containing only ½ per cent. of agar. Obligate aerobes grow only on the surface and about ¼ inch below; facultative anerobes grow down the full line of the stab; while obligate anerobes grow along the line of the stab up to about ½ inch from the top.

E. The *fermentative properties* of the bacteria were tested by inoculating the organisms into a medium consisting of sugar-free meat-infusion standard broth containing 1% of a particular carbohydrate. These cultures were then incubated for 3-5 days and the yield of acid determined by titrating with a *n*/20 solution of sodium hydroxid, with phenolthalein as the indicator. A control un-inoculated tube was also titrated and the difference in acidity between this and the culture fluid gave the value for the acid produced by the bacteria. For most of the organisms the three carbohydrates, glucose, lactose and sucrose, were used. For the streptococci, mannite, raffinose, salicin and inulin were also used. In some cases maltose was included among the test carbohydrates.

F. The *action on milk* was determined in the usual way by inoculating skimmed sterile milk with the culture to be tested. One c.c. of a 1% solution of azolitmin was added to each 10 c.c. of milk to indicate acidity or alkalinity. Acid-production, clot-formation and digestion were noted. Not all the cultures were

subjected to this test. On the whole very little information was obtained from it that could not be gotten from the other tests.

G. *Liquefaction of gelatin* was determined by inoculating the surface of straight gelatin-tubes 1 cm. in diameter, containing 7-8 c.c. of gelatin, and measuring the number of cubic centimeters liquefied at the end of 20 days.

H. *Action on peptone.* (a) The production of ammonia was tested by the Nessler method; (b) indol production was determined with the Ehrlich reagent (*p*-di-methyl amino-benzaldehyde and hydrochloric acid.)

I. *Nitrate reduction* was ascertained by testing for nitrites, in a five-day culture, with naphthylamin hydrochlorid and sulphanilic acid.

The extent of this part of the investigation (A-I) may be surmised from the fact that over 600 strains were isolated, of which about 400 were studied in some detail. A number of the more delicate types not readily adaptable to artificial media died soon after the initial cultivation and only a few of their characters could be ascertained. Others became adapted to artificial existence and were studied more extensively. A detailed characterization of these types will be given later. At this point mention will be made only of some of their general characteristics in order to bring out their relationships to one another, and their occurrence and predominance under certain conditions.

5. **The Frequency of Occurrence and the Abundance of the Various Types of Bacteria under the Different Conditions Studied.**—GENERAL FACTS. From the various investigations summarized in the second paper of this series it was apparent that no definite statement could be made regarding the relative abundance of certain types of bacteria in the normal mouth. On the whole, however, the authors that were quoted agreed on the fact that the cocci are the predominant types, and are always present even in suckling infants. There is also general agreement that thread-forms were found in the mouths of all or almost all the individuals examined, though we can gather little regarding their abundance. Pickerill's and Oshima's results are probably the most reliable, but both are based on smear-examinations.

Tables XI, XII, XIII and XIV indicate the frequency of

TABLE X.—AVERAGE VALUES IN THE COUNTS FOR THE DIFFERENT GROUPS (SEE CHARTS A AND B) OF TEETH REFERRED TO IN TABLES I-IX.

Group	No. of cases in each group	Description of Group	Microscope-count. Average No. per mgm.	Plate-count. No. per mgm.				Ratio * between microscope- and plate-counts
				Aerobic		Anerobic		
				Agar	Glucose-agar	Krumwiede	Jar	
1	2	Immune cases	10,500,000	275,000	550,000	1,320,000	300,000	6:1
2	8	Healthy teeth; "clean" mouths	24,500,000	650,000	990,000	835,000	435,000	13:1
3	2	Healthy teeth; "dirty" mouths	47,000,000	1,380,000	1,860,000	2,050,000	1,000,000	12:1
(3,a)	2	Healthy teeth; "dirty" mouths; tobacco chewers	25,000,000	245,000	335,000	325,000	140,000	38:1
4	3	Healthy teeth, before and after brushing	68,000,000	10,900,000	7,400,000	7,370,000	4,520,000	4:1
			16,250,000	1,700,000	2,300,000	2,000,000	750,000	4:1
5	3	Healthy teeth, before and after meal	26,500,000	124,000	152,000	342,000	278,000	60:1
			67,500,000	5,830,000	2,720,000	3,100,000	4,160,000	7;1
6	3	Primary caries	530,000,000	8,050,000	9,200,000	11,600,000	54,000,000	25:1†
7	5	Caries; enamel and dentin	258,900,000	1,380,000	3,130,000	1,750,000	5,170,000	31:1
(9,a)	3	Pulp exposed; superficial layer	37,200,000	1,480,000	3,900,000	4,420,000	.....	45:1
9	9	Pulp putrescent, deep layer. 3rd and 4th stage of decay	57,670,000	1,270,000	1,060,000	5,010,000	2,670,000	9:1

* In obtaining the ratio the plate-count used is the sum of the aerobic and anerobic counts on the glucose-agar plates.

† If the jar-count were used the ratio would be 9:1, but this jar-count seems aberrant and the Krumwiede plate-count is used, as in all the other cases.

THE LENGTH OF EACH VERTICAL BAR CORRESPONDS TO THE NUMBER OF BACTERIA PER MILLI-
GRAM OF DENTAL DEPOSIT, AS ASCERTAINED FOR A SINGLE SPECIMEN

TABLE XIII.—PERCENTAGE OF EACH TYPE OF ISOLATED BACTERIA IN EACH SPECIMEN EXAMINED.

No. of Specimen	Total number of cultures isolated	Percentage of Gram-positive bacteria	Cocci				Bacilli			Trichomycetes		
			Strepto-cocci	Diplococci		Staphylo-cocci	Aerobic spore-formers	Anerobic spore-formers	Non-spore formers	Branching threads	Non-branching threads	
				Gram-positive	Gram-negative						Short	Long
1	10	60	70	0	10	10	0	0	0	0	10	0
2	12	50	33	17	8.5	8 5	17	0	0	0	17	0
3	7	57	43	0	14	29	0	0	0	0	14	0
4	16	75	44	19	6	19	12	0	0	0	0	0
5	20	60	45	15	10	10	10	0	5	5	0	0
6	20	60	45	0	15	5	10	0	0	0	5	0
7	27	56	37	11	18	4	11	0	4	4	7	4
8	19	64	26	31	16	5	5	11	5	0	0	0
9	20	62	45	10	10	5	5	0	5	0	20	0
10	20	50	30	15	15	5	20	0	5	0	10	0
11	11	50	18	19	27	0	18	0	9	0	18	0
12	10	57	30	30	20	10	0	0	0	0	10	0
13	16	71	44	12	6	6	0	0	19	0	12	0
14	16	63	25	13	13	31	6	0	6	6	0	0
15	18	64	44	17	17	0	17	5	0	0	0	0
16	20	60	20	5	25	0	10	0	20	5	15	0
17	22	50	32	5	27	9	9	0	9	0	9	0
18	15	55	27	7	20	6	13	0	6	7	13	0
19	20	70	30	10	10	10	10	5	0	0	20	5
20	14	66	43	0	7	14	7	0	7	0	14	0
21	16	55	38	12	25	0	6	0	6	0	13	0
22	16	50	25	19	12	0	0	19	25	0	0	0
23	18	38	17	11	11	11	11	17	22	0	0	0
24	18	60	11	6	22	0	0	22	39	0	0	0
25	8	50	0	12	37	0	0	13	38	0	0	0
26	16	40	31	12	0	0	6	25	12	0	6	1 yeast
27	20	70	25	0	10	0	5	10	25	0	20	5
28	20	50	30	0	10	0	5	0	25	5	15	10
29	20	63	20	10	10	10	10	5	35	0	0	0

30	24	71	21	9	17	8	8	0	12	4	13	8
31	16	57	44	6	13	0	0	6	12	0	19	0
32	16	88	6	0	19	6	6	0	25	0	25	13
33	9	60	22	11	33	7	0	0	34	0	0	0
34	15	70	20	0	0	0	13	0	40	0	7	13
35	13	71	23	0	15	0	15	0	47	0	0	0
36	13	73	23	15	7	0	7	7	30	0	7	0
37	16	91	25	13	12	0	0	0	25	0	25	0
38	10	100	20	0	0	0	10	0	20	10	30	10
39	16	86	12	13	6	0	0	0	37	0	25	6
40	15	79	33	7	7	6	0	0	20	7	13	7

TABLE XIV (SEE CHART D).—PERCENTAGE OF EACH TYPE OF ISOLATED BACTERIA FOR EACH OF THE NINE GROUPS OF CASES.

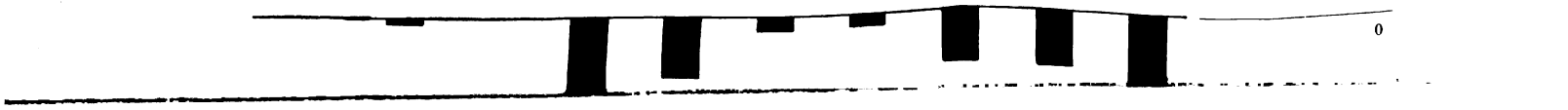
No. of group	No. of cases	Total no. of cultures isolated	Description of group	Cocci				Bacilli			Trichomycetes		
				Streptococci	Diplococci		Staphylococci	Spore-formers		Non-spore-formers	Branching threads	Non-branching threads	
					Gram-positive	Gram-negative		Aerobes	Anerobes			Short	Long
1	2	27	Immune cases...	44	7	11	11	4	0	4	0	15	0
2	8	113	Healthy teeth; "clean" mouths	39	14	13	10	9	1	5	2	7	0
3	4	83	Healthy teeth; "dirty" mouths	43	11	15	7	13	0	2	2	6	2
4	3	57	Healthy teeth; before brushing.	26	5	25	5	10	0	12	4	12	0
5	3	50	Healthy teeth; after meal.....	36	8	14	8	10	2	4	0	16	2
6	3	60	Primary caries...	20	13	15	5	7	0	20	3	16	10
7	4	41	Caries; enamel and dentin.....	22	7	5	2	2	0	27	5	22	7
8	4	71	Pulp exposed....	27	4	8	4	7	5	28	0	11	4
9	7	105	Pulp putrescent.	21	15	14	3	4	17	23	0	2	0

condition in any other part of the mouth, the results for such cases would have been even more striking in this connection. The significance and possible interpretation of these results will be discussed more fully later, this section being devoted primarily to the presentation of the actual data.

The occurrence of the trichomycetes is also suggestive. Branched forms were found regularly in the first and second stages of decay and occasionally in the normal cases. The short threads were found under all conditions, though more frequently in the early stages of decay, on the unbrushed teeth and after the meal. The long threads (or what I believe are the true *Leptothriceae*) are found only rarely, if at all, under any condition other than those of the first and second stages of caries, in which they occur in large numbers. In decay of the pulp all of these forms are absent.

On examining Tables XIII and XIV, which show in a general way the abundance of these types under different conditions, further light is obtained on the possible relationship of these types to the process of decay in its different stages. Thus, of the total number of strains isolated in Group 1, 44 per cent. were streptococci and over 70 per cent. were cocci of some sort, while only 8 per cent. were bacilli and 15 per cent trichomycetes. This general ratio is noted also in Groups 2, 3, 4 and 5, about 70 per cent. of the strains being cocci and only 30 per cent. representatives of the other two families. Anerobic bacilli are absent and non-spore-formers occur only in small numbers, the same being true of the threads.

The ratio is decidedly different for the groups representing the specimens taken from diseased teeth. The percentage of cocci drops to between 40 per cent. and 50 per cent.; that of streptococci alone from about 40 per cent. to 20 per cent., a decrease of one-half; while there is a corresponding increase in the numbers of non-spore-bearing rods and of thread forms. In Groups 6 and 7 (consisting of cases of first and second stages of decay), the thread-forms rise to about 30 per cent., an average increase of 100 per cent. to 200 per cent.; while there is proportionately an even greater increase in the non-spore-bearing bacilli. These rods belong to the Gram-positive acidific bacilli, which are facultative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anerobes and active acid-producers, fermenting both glucose and lactose with great avidity.

Group 8 is the transitional group mentioned before. The striking results here are the presence of a small percentage of anerobic spore-formers and a drop in the percentage of thread-forms. Both the types characteristic of Groups 5 and 6 and those of Group 9, respectively, occur in this group though in decreased abundance.

Group 9 is distinguished from the others by practically complete disappearance of the thread-forms and by marked increase in the percentage of anerobic spore-formers.

CORRELATION OF THE DATA. The results presented above, when correlated with one another, exhibit a marked coherence. This agreement is especially prominent for the diseased teeth where the division into groups is based on the clinical diagnosis of the single tooth from which the specimen was taken and not on the condition of the whole mouth, as in the case of the specimens from normal teeth. In the one case the basis is specific and definite, and less liable to wide fluctuations, while in the other the basis is more general and therefore more prone to include complicating factors that may lead to discrepancies. The general consistency of the results justifies, however, the division made, though further study will most probably lead to modifications.

On comparing the results obtained in Groups 1, 2 and 3, respectively, it is evident that the flora is generally the same in character in all of them, the streptococci and cocci as a whole predominating and occurring with greater frequency than do the other types. The prevailing flora on the *healthy* teeth, whatever the condition of the mouth may be, is approximately the same in all cases. That there is a decided difference in the environment which each individual presents for the development of these types is evidenced by the greater number of bacteria found in one group than in another. In fact, this is the only index of the existence of such a difference. That conditions in the mouth exert marked influences on the growth of oral bacteria is further shown by the large increase in the total number of bacteria during the night, and during and immediately following a meal. The general relationship of the types to one another is altered

only slightly, however, the shift being in the decrease of the cocci and the increase of the forms characterizing early stages of decay. The results of the study of bacteria of healthy teeth indicates, on the whole, that, while there is rise and fall in the total numbers of bacteria with the changing conditions in the mouth, the types of organisms and their *relative* abundance remain fairly constant.

A totally different conclusion results from a correlation of the data obtained from a study of *diseased* teeth. Here too there is a decided difference in the total numbers of bacteria present at the different stages or degrees of decay. Associated with this difference there is, however, not only a complete change in the character of the flora and the relative prevalence of types from that of the healthy teeth; but there is also a distinct difference between the types of bacteria in the early stages and those in the later periods of decay.

Primary caries may, from the enormous counts and from the changed character of the prevailing flora, be considered a specific infection in which a limited number of types (perhaps three) are concerned. Just what is the predisposing factor or which of these types is most instrumental in bringing about decay, or whether we do not deal here with a true association of types, is hard to say. The significant facts are the (a) marked increase in the acidific bacilli, some of which are capable of producing and resisting an acidity of 8 per cent. *N* acid, and the (b) accompanying numerical increase in the long and short thread-forms, which can readily attach themselves in the form of compact colonies to any surface. One type of pleomorphic short thread-forming organism, growing in comparatively large colonies, has been observed regularly to attach itself to solid glass surfaces and to the wall of the test tube in liquid cultures, while in plate colonies it was often found enclosing one or two colonies of other bacteria. This type is relatively abundant in carious teeth. The relation this organism may bear to the concentration on a small area of a large number of active acid-producing rods is suggestive to say the least.

The decrease in the number of streptococci at all stages of decay fails to support the prevailing view [Goadby, Sieberth (9),

Kantorowicz (14a), Baumgartner (3), *et al*] that they are the important agent in caries. The foregoing evidence on this point is circumstantial and does not warrant any conclusions that step beyond the limits of the facts; but it is undoubtedly stronger than any proof that has hitherto been presented in favor of recognizing the streptococci or any other organisms as the causative agents in dental decay. For the present it is clear that the early stages of caries are bacteriologically due to one and the same process, characterized by a great increase in the total number of bacteria, accompanied by a drop in the relative number of cocci, and a marked increase in the number of acidific bacilli and thread-forming organisms.

Goadby (9) has observed the presence, in almost pure cultures, of an organism, from the deep layers of dentin, closely resembling the acidific bacillus and named by him *B. necrodentalis;* but he never associated decay of enamel and dentin with this form. Leber and Rottenstein, the pioneers in this field, long ago attributed caries to what they termed "leptothrices." The lack of correlation as well as the usual abundance of streptococci resulted in focusing attention on the latter as the etiologic agents of decay.

Decay of the pulp, as noted from a correlation of the results in group 9, is a process that is different in character from decay of enamel and dentin. Associated in this process are a (a) relatively low bacterial count, a (b) drop in the relative number of cocci, similar to that found in primary decay but differing from the latter also in showing a disappearance of the thread-forms, and the (c) presence in all cases of anerobic putrefying bacilli. The acidific type persists in practically the same abundance as in the initial stages of decay.

Here again it is difficult to assign with certainty to any particular form the role of furthering pulp decay. Various authors have invoked diverse agencies to explain this phenomenon. Kantorowicz (14) ascribes the process to the action of leucocytic enzymes, whereas Goadby (9) attributes it to the proteolytic enzymes of the bacteria on the decaying surface of the tooth. Most authors agree that decay of the pulp is attributable to bacterial enzymes. There is no doubt that the anerobic spore-

formers, which possess powerful proteolytic properties and are found in decay of the pulp and under no other condition, might be the agents concerned in initiating and completing this process. Rodella (28a) was the first to call attention to this organism in connection with dental caries and went so far as to attribute to it the process of decay from start to finish. My results in this connection are so striking that the conclusion seems to be warranted, that the anerobic putrefying bacillus (*B. putrificus*) is closely related to the process of pulp decay.

6. **Description of Types.**—GENUS STREPTOCOCCUS. The classification of this group has been a bone of contention for the last 15 years. More recently Gordon (10, 11), and Andrewes and Horder (1) in England, Winslow (33), Hilliard (30), Hopkins and Lang (13), Broadhurst (5), Lyall (22), Kligler (17), and others in this country, have suggested a subdivision of this genus based largely on the fermentative action of the different strains on glucose, lactose, sucrose, salicin, mannite, raffinose and inulin. These authors agree that the organisms belonging to the type *Str. pyogenes* ferment glucose, lactose, sucrose and salicin, but not the other substances. There is also fair agreement that the *Str. salivarius* (or *Str. viridans*) ferments these four substances and also raffinose, but usually not mannite. The type fermenting mannite and not raffinose is classed as *Str. fecalis.* No particular name has yet been given to the inulin fermenters, which abound in the human mouth and, contrary to the general belief, are not pneumococci.

In this investigation 123 strains of streptococci, of which 17 gave the morphology of pneumococci, were studied in detail. The grouping of these strains is summarized in the accompanying table (XV).

The results, as shown in Table XV, correspond in a general way with those obtained by Hilliard in his study of the throat streptococci. His percentages are 16.7 per cent for the glucose-lactose-sucrose group; 7.5 per cent. for the glucose-lactose-sucrose-salicin group; 23.8 per cent. for the raffinose and 6.7 per cent. for the inulin groups, respectively. The only very marked differences are noted among the sucrose and inulin fermenters. In the former group are included, however, strains which died

TABLE XV.—DATA PERTAINING TO FERMENTATIVE TYPES OF STREPTOCOCCI ISOLATED FROM DEPOSITS ON NORMAL AND DECAYED TEETH.

Substances Fermented	Glucose, Lactose	Glucose, Lactose, Sucrose	Glucose, Lactose, Sucrose, Salicin	Glucose, Lactose, Sucrose, Salicin, Raffinose	Inulin *	Mannite **	Positive	
							Gram	Milk
No. of strains....	13	36	11	40	27	6	94	63
Percentage of total number of strains tested..	11	30	9	32	22	4	85	90

* The mannite fermenters did not all attack salicin; 5 out of the 6 failed to ferment raffinose.

** Some of the inulin fermenters attacked raffinose, some mannite and some failed to ferment either. It was thought best to group them all as inulin fermenters.

before they could be tested on the other substances, resulting in a higher percentage; while the inulin group contains ten strains that gave a typical pneumococcus morphology. On the whole, therefore, there is little difference between the streptococci found on teeth and those in other parts of the oral cavity. Pyogenic streptococci are encountered only infrequently. Saprophytic strains, corresponding with the largest group found in milk, are quite abundant, while mannite fermenters (*fecalis* type) are rare.

The two remaining large groups are of special interest. The raffinose-fermenting type is most frequently found and is the one which usually occurs in subacute infections, such as endocarditis and rheumatism, and in pyorrhoea alveolaris (12). The inulin-fermenting types are of interest because inulin is considered diagnostic of pneumococci. A comparatively large group of mouth streptococci respond to that test.

The *cultural features of the streptococci* are the same for all types and may be briefly stated, as follows.

Morphology.—The typical organism is a spherical or somewhat oval cell occuring in chains composed of from a few to a very large number of elements. The length of the chains, as well as the size of the individual cells, varies considerably with differences in the culture medium. Division is always in one plane and packet-forms or clumps are rarely, if ever, seen. *Chromology.*—They usually stain readily with all anilin dyes, and most often retain the dye when stained by Gram's method. *Cultural characters.*

—Upon ordinary solid media, such as *agar* and *gelatin,* only a faint, veil-like growth of minute discrete colonies occurs. Addition of glucose, and especially of animal serum, is favorable to their development. *Gelatin* is not liquefied by the common types. In *broth,* a fine or coarse flocculent sediment is usually obtained. None of these characters is of value in the differentiation of the various types, though they are all distinctive for the group. The growth on blood-agar is often used to distinguish the different varieties. The pyogenic cocci usually give a clear zone of hemolysis, the pneumococci and mildly pathogenic streptococci produce green colonies, whereas the saprophytic usually do not affect the blood at all. The distinction is not, however, sharp enough to serve as a basis for a fundamental differentiation of the streptococci. The fermentation reactions have proved more reliable for that purpose; these reactions are indicated in the accompanying table (XVI).

TABLE XVI.—DATA PERTAINING TO FERMENTATION-REACTIONS OF STREPTOCOCCI ISOLATED FROM DEPOSITS ON NORMAL AND DECAYED TEETH.

Type	No. of Strains	Glucose	Lactose	Sucrose	Salicin	Raffinose	Mannite	Inulin
Str. I.....	13	+	+	?	..	..	..	..
Str. anginosus....	36	+	+	+	−	−	−	−
Str. pyogenes.....	11	+	+	+	+	−	−	−
Str. salivarius.....	40	+	+	+	+	+	−	−
Str. II.....	17	+	+	+	±	±	±	+
Str. fecalis.....	6	+	+	+	+	−	+	−
Pneumococcus....	10	+	+	+	±	±	±	+

Summarizing the results with this group, it is evident that *Str. anginosus* (Andrewes and Horder) and *Str. salivarius* (or *Str. viridans*) [Andrewes and Horder] are the two species most abundantly present in normal as well as carious teeth; and, according to Hilliard, these are also the common types in the human throat.

GENUS DIPLOCOCCUS Pneumococci were found occasionally and, because of their infrequent occurrence and their close relationship to the streptococci, were included with that genus. The

diplococci did not occur so abundantly as the streptococci, though they appeared in almost every specimen. In all, 57 strains were studied. Of these, 53 were Gram-negative and only 4 were Gram-positive. The Gram-positive varieties gave good whitish growth on agar-streaks; fermented glucose, sucrose and lactose; but failed to liquefy gelatin or produce indol. Their systematic position is not clear; they are apparently closely related to the albococci.

The Gram-negative strains fall into four divisions according to their fermentation reactions; 8 failed to ferment any of the sugars; 6 fermented glucose but did not attack lactose or sucrose; 34 fermented glucose and sucrose but not lactose; and 5 fermented the three sugars. Morphologically these strains are all more or less alike, resembling very closely the meningococcus. Culturally it is equally difficult to differentiate them. The non-fermenters are most probably *D. catarrhalis,* those fermenting all the test sugars belong to *D. crassus,* while those attacking glucose and sucrose and those attacking glucose only, are undoubtedly the chromogenic groups I and II, respectively, of Elser and Huntoon, or *Diplococcus flavus* II and I, respectively, of Lingelsheim.

The most prominent group, often abundantly present, is the one that ferments glucose and sucrose. This diplococcus is found so regularly and often, in such great numbers, that is must be considered a normal inhabitant on the teeth. It will suffice to give the cultural characters of this form as a type, since there is little difference between the various species. Besides, these organisms have been studied and described in detail by Elser and Huntoon (7), and Lingelsheim (20). The reason for repeating the description is the fact that none of these organisms has been considered a characteristic oral type. My findings indicate, however, that they are normal inhabitants of the oral cavity. Elser and Huntoon (17) have found Gram-negative cocci regularly in the respiratory passages of nursing infants and of older children.

Morphology.—The typical organism (*Diplococcus flavus*) is a small biscuit-shaped coccus occuring in twos and often in large clumps. Chains are never seen. There is some variation in size depending on the medium used. The cells are usually smaller

than the meningococcus. *Chromology.*—They stain readily with ordinary dyes, but fail to stain by Gram's method. *Cultural characters.*—In liquid cultures they show little turbidity, usually sedimenting to the bottom. The *flavus* types often color the medium a greenish yellow, especially in old cultures. On solid media they usually appear as small, raised, lustrous, whitish or yellowish colonies from 0.5 to 2 mm. in diameter. Microscopically the colonies have a regular, flat edge, the center appearing to be filled with dark coarse granules. On a slant the colonies flow together after 2-4 days, giving a lustrous yellowish to yellow growth. In older cultures this becomes brittle and crumples up readily when tested with a needle. They grow readily on agar but somewhat better on glucose- and serum-agar. Their viability on any medium is not very great. Most of them live only 1-2 weeks, especially shortly after isolation.

The only marked cultural differences among the various types is the size and color of the colonies. The *catarrhalis* colony is usually smaller than the *flavus* and greyish white in color, while the *crassus* colony is much like the *catarrhalis* but less brittle. The differentiating fermentative characters are shown in the accompanying table (XVII).

TABLE XVII.—DATA PERTAINING TO FERMENTATION-REACTIONS OF GRAM-NEGATIVE DIPLOCOCCI ISOLATED FROM DEPOSITS ON NORMAL AND DECAYED TEETH.

Type	No. of Strains	Glucose	Lactose	Sucrose	Gelatin	Milk	Indol
D. crassus	6	+	+	+	−	+	−
**D. flavus, II*	33	+	−	+	−	−	−
**D. flavus, I*	5	+	−	−	−	−	−
D. catarrhalis	8	−	−	−	−	−	−

*A number of the *flavus* strains were also tested on maltose and found to give positive results.

STAPHYLOCOCCI. The staphylococci have been studied extensively by Gordon (11) in England, and by the Winslows (34) in this country. Gordon paid special attention to the white cocci occurring on the different parts of the body; the Winslows in-

cluded in their investigation all varieties of cocci. As a result of their work, the Winslows divide the abundantly growing staphylococci into four genera according to the type of pigment produced, and other correlated characters. The validity of this classification was confirmed by me in a previous study (18).

The cocci most often found on the teeth in the present series of observations were of the white variety, belonging to the genus *Albococcus* (Winslow). Of the 29 strains studied, 27 were albococci and only two belonged to the genus *Aurococcus*. Of the 27 white cocci, 21 were non-liquefiers; 6 liquefied gelatin, 3 of the latter reducing nitrates.

Using the classification of Gordon and Winslow, the form most frequently found on the teeth was the *Alb. candidus*, while the *Alb. pyogenes* and the *Alb. epidermidis* were represented by only 3 strains each. The two aurococci fermented glucose, lactose and sucrose but failed to liquefy gelatin. It is of interest to note that the non-liquefying cocci predominate on the teeth, and that the orange cocci are practically absent.

The characteristics of the staphylococci are well known, but for the sake of completeness the general cultural and morphological properties are briefly summarized below.

Morphology.—The cells (*Genus Albococcus*) are generally round and aggregated in irregular masses in one plane. The diameter is about 1 μ, with slight variations. *Chromology.*—They stain readily with anilin dyes and usually retain the Gram-stain. *Cultural characters.*—The white cocci give abundant grayish white to lustrous white growth on agar and gelatin. Liquefaction of gelatin, fermentation reactions and reduction of nitrates usually serve to differentiate them into specific types.

The aurococci are culturally much like the albococci except that they produce an orange pigment on various culture media, do not grow as abundantly on agar, grow much better at 37° C than at 20° C, generally reduce nitrates, and liquefy gelatin rapidly. (See Table XVIII).

GENUS BACILLUS. This genus includes a large number of bacteria of different types totally unrelated to one another except that they are all rod-shaped. I have therefore divided the organisms of this family into three large groups and will consider them under those heads.

TABLE XVIII.—DATA PERTAINING TO FERMENTATION-REACTIONS OF STAPHYLOCOCCI ISOLATED FROM DEPOSITS ON NORMAL AND DECAYED TEETH.

Type	No. of strains	Glucose	Lactose	Sucrose	Gelatin	Indol	Nitrate	Gram
Alb. candidus.....	21	+	+	+	−	−	−	+
Alb. pyogenes.....	3	+	+	+	+	−	−	+
Alb. epidermidis..	3	+	+	+	+	−	+	+
Aur. mollis.....	2	+	+	+	−	−	+	+

A. GROUP OF AEROBIC SPORE-FORMING BACILLI[4].—The aerobic spore-formers have not as yet been satisfactorily differentiated into specific types. I shall therefore give only the general characters of the group and refer for details to standard books like those of Chester (6) and Migula (23), and especially Goadby (9), where full descriptions of the aerobic spore-formers that occur in the mouth will be found. The organisms of this type usually belong to the mesentericus group, though a large list of names which are undoubtedly synonyms have been used to differentiate them. Thus, there are the *B. gingivae pyogenes* and *B. pulpae pyogenes* (Miller), the *B. gangrenae pulpae* (Arkovy), the *mesentericus vulgatus, ruber* and *fuscus,* respectively, and so on. I have been able to identify only two groups among the isolated organisms. By far the greater number were of the mesentericus variety.

(a) *Mesentericus group. Morphology.* — Long, round ended rods, 0.7-1 μ. wide and 2-4 μ long; often long threads; form large oval spores. *Chromology.*—Stain well with ordinary stains and with Gram's. *Cultural characters.*—On agar a thick, dry, corrugated, greyish growth is obtained in 18-24 hours. Gelatin is rapidly liquefied. On broth a pellicle is formed.

[4] Since this paper went to print, Maher published an article in the *Medical Record* (1915, vol. 88, p. 89), in which he attempts to show that all organisms are but modified descendants of spore-forming bacilli. In this connection he states that although he made many hundreds of cultures from human sputum, he was never able to grow spore-bearing bacilli in any kind of culture media, solid or liquid. This finding disagrees with those of all other workers in this field. While I have not been able to find spore-formers as frequently, or as abundantly, as did previous observers, I have isolated them in quite a number of cases.

Glucose, lactose and sucrose are fermented. Milk is clotted and digested. Indol and ammonia are produced in peptone-water. The organisms are aerobic but they grow (though less readily) under anerobic conditions.

(b) *Subtilis (?) group. Morphology.*—This type is characterized by a short, thick, square-ended rod 1 x 2 μ. It is actively motile and produces large oval spores. *Chromology.*—It takes all stains readily, but reacts variably to the Gram. *Cultural characters.*—This bacillus is strictly aerobic, gives a brownish spreading growth on agar, liquefies gelatin and digests milk. Glucose is fermented but lactose and sucrose are usually not attacked. It produces ammonia but no indol. It is thus in every way sharply differentiated from the mesentericus group. It was found on normal but not on decayed teeth, while the mesentericus was found on both.

(c) *B. maximus.* This organism has been described by Miller and others, but was first grown in pure culture by Goadby. It does not grow rapidly on ordinary media when first isolated but can be readily adapted to artificial environments. I have isolated only three strains and those from " dirty " normal teeth. They agreed in all essentials with the type described by Goadby.

Morphology.—Long, thick bacillus; often chains and long, winding threads. It is non-motile and forms spores. *Chromology.*—Young cultures stain readily with the anilin dyes and by Gram. Older cultures stain irregularly and decolorize by Gram. *Cultural characters.*—On agar: small, round, raised, brownish colonies. On the streak they grow as discrete colonies that flow together somewhat, giving a granular, lustrous, brownish growth. Gelatin is liquefied slowly. In broth, growth is scant. Glucose and lactose are fermented. No indol or ammonia is produced. Growth is better at 37° C. than at 20° C.

B. ANEROBIC SPORE-FORMING BACILLI. Fifteen strains belonging to this group have been studied. Judging from their various characters, all belong to the same type—*B. putrificus* of Bienstock (4). They resemble the organism found by Rodella (28a) though their qualities do not wholly agree with the incomplete description given by him.

(a) *B. putrificus. Morphology.*—A long, thin rod 0.6 μ. x

4-8 μ. *Chromology.*—Stains readily with ordinary stains but is decolorized by Gram's method. In this respect it differs from the property ascribed by Rodella, whose organism was Gram-positive. All my 15 strains were Gram-negative. The rod varied in length and thickness according to the age of the culture and nature of the medium, but these variations were noted in the same strain as well as in the different strains. *Biological properties.*—The bacillus is motile, strictly anerobic, and forms oval-end spores. It closely resembles the tetanus bacillus, except for the fact that its spore is oval whereas that of the latter is round. Rodella claims to have observed a central spore, an observation also recorded by Bienstock (4). I have not seen spores anywhere else than at the end and am of the opinion that Tisier (31) was correct in his description of this organism. Spores are generally formed after 48 hours. *Cultural characters.*—In agar-stabs the growth has a fluffy appearance, which is more marked in glucose-agar. In glucose-agar, gas is usually produced. Gelatin is liquefied. The growth in the gelatin-stab is much like that in agar. In broth and sugar-broth a heavy uniform turbidity is produced. Glucose and lactose are regularly fermented, while sucrose is feebly acted on by some and not at all by others. Milk is digested with a final acid reaction. Indol and ammonia were produced by all, nitrates were reduced by only a few. The various properties are summarized in Table XIX.

TABLE XIX.—DATA PERTAINING TO THE B. PUTRIFICUS ISOLATED FROM DECAYED PULP.

Type	No. of strains	Gram	Spores	Glucose	Lactose	Sucrose	Gelatin	Indol	Ammonia	Nitrates	Casein
B. putrificus, I.....	8	−	+	+	+	+	+	+	+	−	+
B. putrificus, II.....	9	−	+	+	+	−	+	+	+		+

C. NON-SPORE-BEARING BACILLI. The third group of bacilli consisted almost entirely of a single type. There were two or three colon-like organisms, a few diphtheroids and two strains of the Friedlander bacillus; but the majority belonged to the group

of Gram-positive, actively acid-producing rods. In this division there were two distinct organisms: one a thin pleomorphic rod readily adapted to ordinary media; the other a thick, non-pleomorphic, beaded bacillus which could not be grown for more than two or three generations on artificial media. The former was observed most frequently in the material obtained from carious teeth, the latter in that from normal teeth. Of the former about 60 strains were studied; of the latter, only five could be kept alive sufficiently long to ascertain some of its peculiarities.

The pleomorphic bacillus undoubtedly belongs to the acidophilus or acidific group of organisms found in human and animal feces, first by Moro (25) and later by Finkelstein (8), Rodella (28), Mereschkowsky (24), Kendall (15), and others. Goadby (9) describes an organism isolated by him from the deep layers of decayed dentin which agrees in most particulars with this type.

In this investigation 58 strains, isolated from material at the different stages of decay, were reserved for detailed study. The organisms were highly pleomorphic but constituted, on the whole, a single homogeneous group, which could be separated into two varieties by differences in their fermentive actions on sucrose.

(a) *B. acidophilus (Moro). Morphology.*—The bacillus is highly pleomorphic, varying from a uniform, small, colon-like rod to long winding threads. On agar streaks it is usually 0.6 x 1.5-2.0 μ and occurs in twos, often in long chains. Under anerobic conditions the rods are longer and frequently filamentous. *Chromology.*—Young cultures stain readily though faintly with the ordinary dyes and retain the stain when treated by Gram's method. *Biological properties.*—It is a facultative anerobe, grows better under anerobic conditions when first isolated, is not motile, does not form spores, and grows better at 37° C. than at 20° C. Cultures recently isolated should be transferred every 10 days or so. Cultures that have been adapted to artificial media live much longer. *Cultural characters.*—It can be best isolated on media containing glucose and made sufficiently acid preferably with acetic acid to inhibit the growth of other organisms (an acidity of 3-4 per cent. *N* acid is most satisfactory). It may also be readily obtained from glucose-agar or glucose-broth which has

been incubated for about 5 days to a week, the marked resultant acidity generally killing off the other organisms and the *acidophilus* being obtained in pure culture in the second or third subculture in glucose media.

On agar and glucose-agar the colonies are very small, usually not more than 0.5-1 mm. in diameter. Microscopically they show a dark center with radiating strands. Glucose-agar is always rendered distinctly turbid. Gelatin is not liquefied. In broth there is a heavy sediment with little turbidity. Milk is usually, though not always, clotted, the lower portion being coagulated first. Litmus-milk is decolorized. Glucose is readily fermented with the production of a large amount of acid, often equivalent to about 7-8 c.c. of *N* sodium hydroxid per 100 c.c. of medium. Lactose is fermented but not attacked with the same avidity. Sucrose is readily fermented by some but not by others; maltose is broken down by all. Neither ammonia nor indol is produced in a peptone solution. The salient characters are summarized in the accompanying Table.

TABLE XX.—DATA PERTAINING TO B. ACIDOPHILUS ISOLATED FROM DEPOSITS ON NORMAL AND DECAYED TEETH.

No. of strains	Gram	Glucose	Lactose	Sucrose	Milk: Clot	Litmus milk: Reduction	Gelatin	Indol	Ammonia
3	+	+	−	−	−	−	−	−	−
35	+	+	+	−	+	+	−	−	−
20	+	+	+	+	+	+	−	−	−

(b) The *non-pleomorphic beaded bacillus* has not been described before as far as I was able to ascertain. In a recent paper Serkowski (29) describes as a new species an organism resembling this bacillus, which he isolated from nasal secretion, and from the bladder in rhinitis and cystitis, respectively, and from the normal mucus of the conjunctiva, nose and mouth. I am not sure, however, whether the two organisms are identical.

Morphology.—A medium thick rod 1 x 3 μ. *Chromology.*—Stains readily with ordinary dyes, giving a beaded appearance

(usually two or three beads) with gentian violet and a granular, structure like that of the diphtheria bacillus when stained with methylene blue. The number of granules correspond with the number of beads. It is Gram-positive. *Biological properties.*—The bacillus is a facultative anerobe; non-spore-forming, non-motile; grows better at 37° C. than at 20° C. and is not readily adapted to artificial cultivation. This last property distinguishes it from the *B. granulobacillus* of Serkowski. *Cultural characters.*—On agar plates the colonies are very small, 0.5 to 1 m.m. in diameter, white, round and slightly raised. Anerobically, the growth is in the form of small spherical discrete colonies. Gelatin is not liquefied. Glucose and lactose are fermented and milk clotted. Sucrose is usually not fermented.

For the present, until a further study of this form may definitely establish its relationship to the *granulobacillus* of Serkowski, that name may be applied to this type. I suggest, however, that the crude, unwieldly name *B. granulobacillus putrificus* be modified to *B. granulatus.*

Trichomycetes. The family of filamentous bacteria is indeed puzzling. It is not even clear whether the different members belong to the same phyla. Petruschky (26), in the latest edition of Kolle and Wassermann's work, divides these organisms into two families, the *Trichomycetes* and *Trichobacteria.* The former, including the branched forms, *Actinomyces* and *Streptothrix,* he classes with the *Hyphomycetes;* the latter, consisting of the *Cladothrix* and *Leptothrix,* he groups with the *Schizomycetes.* This division seems to distinguish the natural relationships of these forms but cannot as yet be considered final.

So far the typical mouth representatives of this family have not been successfully grown on artificial culture media. Goadby has isolated and described a species belonging to the genus *Actinomyces.* I have also isolated this type from a number of cases, but it occurs only irregularly and not in sufficient numbers to be found frequently in dilutions above 1:1000. The true, mouth, thread-forms, however, have not hitherto been isolated except perhaps in the single instance reported by Dobrzyniecki.

I have succeeded in cultivating two distinct species, one of which consists of at least two varieties. One type is present

on all kinds of human teeth but is especially abundant in caries of the first and second stages. The other has been found on normal teeth only twice in the higher dilutions, but was very abundant and was isolated regularly from all cases of caries of enamel and dentin. Both species are practically absent in the advanced stages of pulp-decay, and are present in only moderate numbers in its early stages.

The forms most abundantly present in the mouth belong to the non-branching *Trichobacteria*. Whether they are both true *Leptothricæ* is doubtful. Since Miller's day, when differentiation of types was more difficult, there has been a tendency to call everything that looked like a thread, *Leptothrix*. What has been said above, of the pleomorphic thread-forming rods, shows the fallacy of this assumption.

Leptothrix-forms were first detected, under pathological conditions, by Frankel (26), in the follicles of the tonsils and at the base of the tongue. Chiari (26) reported a case of pharyngomycosis which he attributed to *L. buccalis*. Arustamoff (2) is the only one who has apparently succeeded in cultivating a non-branching leptothrix from tonsilar-deposit in two cases of pharyngomycosis, and one from the urine of a patient with tabes. The organism he describes is 0.5-0.6 μ thick and from 8-50 μ long. The one obtained from the urine grew best at 37° C., poorly at 20° C., and grew only in the stab. There was no growth on gelatin and hardly any in broth. The one from the pharynx grew best at 37° C.; grew on the surface, and liquefied gelatin. This is the only authentic though meagre description of the cultural characters of a true *Leptothrix*.

One of the types I have isolated resembles this closely, the other corresponds in a general way with the *L. placoides albus* of Dobrzyniecki and is more probably a *Cladothrix*.. This latter organism will be described first and, since it has some of the general features of the form described by Dobrzyniecki, the name *placoides* is retained, the generic name *Cladothrix* used, and the last part dropped to avoid a trinomial. The name *placoides* is specially appropriate for this organism, because of its property to form plaque-like colonies that cling to surfaces.

(a) *Cladothrix placoides.*—Fifty-eight strains of this or-

ganism were studied. Judged from the growth on agar there were two distinct varieties, one giving a dry, granular, non-lustrous growth on the streak with a raised, non-lustrous, convoluted colony; the other giving a moist, lustrous, grayish-white to white growth, and a round, raised, discrete glistening colony, which sometimes became wrinkled in old cultures. These differences could not, however, be correlated with other morphological and physiological properties. All the strains are, therefore, for the present included in the same group, leaving further differentiation to future study. Of the 58, 42 were Gram-positive, 17 liquefied gelatin, 30 fermented glucose and sucrose, and 17 also fermented lactose. Most of them reduced nitrates, while all failed to produce ammonia or indol.

Morphology.—The morphology of this organism is very variable. It often appears like a coccus, sometimes like a diphtheroid rod, or short club-end thread. Usually one finds all three in the same preparation. The coccus-like forms are probably spores, and the others are pleomorphisms of the thread. Microscopic pictures are often obtained which look identical with *L. innominata* of Miller. *Chromology.*—It stains readily with ordinary anilin dyes and is usually Gram-positive. Often the spores retain the stain while the threads are decolorized when treated by Gram's method. *Biological properties.*—They are aerobic, non-motile, non branching threads. They grow best at 37° C., only very slowly at 20° C. *Cultural characters.*—On agar they form round, raised, lustrous, white colonies which can be picked up entire from the medium. On glucose-agar the colonies are usually larger and more grayish. Microscopically the surface colonies are convoluted and are often seen on the plates enclosing underneath them small colonies of other bacteria. In broth the growth is very sparse, and the wall of the tube is usually covered with a granular layer of discrete colonies 0.5 mm. or less in diameter which are not readily removed by shaking the tube. In glucose-broth the growth is very abundant, giving both a heavy turbidity and a granular deposit on the wall. Gelatin was liquefied by some strains but not by others. They all fermented glucose, most of them fermented sucrose, while some also fermented lactose. Gas is not produced. They generally

reduce nitrates and fail to produce indol or ammonia. When first isolated they should be kept on glucose-agar and transferred every two weeks.

(b) *L. buccalis.* (Miller, Kligler). Fifteen strains of the true *Leptothrix* were studied in detail. They correspond with the description usually given for the typical organism. They all showed the same morphology and resembled microscopically both the *L. buccalis* of Miller and the *L. racemosa* of Vincentini and Williams. Since this organism appears to be the true mouth *Leptothrix,* I shall adopt for it the name *L. buccalis* with the following characterization.

Morphology.—A thick, long, straight, or curved thread with a club-head at one extremity and a tapering end at the other. It is generally 0.8-1 μ thick and upwards of 10 μ in length. *Chromology.*—It stains readily with anilin dyes in young cultures. In older cultures it has the appearance of a faintly stained sheath enclosing a number of heavily stained granules. Young cultures are Gram-positive. In older cultures the sheath is decolorized while the granules retain the stain. It is not pleomorphic but the threads fragment very early into short, thick rods. Coccoidal forms are not seen. *Biological properties.*—They are anerobic, facultative-aerobic, non-motile, non-branching threads. They grow at 37° C. and practically not at all at 20° C. *Cultural characters.*—No growth is obtained on agar. On glucose-agar-plates they give minute pin-point colonies after 3-4 days' incubation. Examined microscopically with a low power, they have a dark center with hairy outgrowth. They grow fairly well in serum-glucose-agar, best in the stab and only sparsely on the surface. The surface colony is raised, round, whitish, lustrous, rarely more than 0.5 mm. in diameter. The addition of salivary mucinate to ordinary agar renders the latter a very favorable medium, a thin, grayish white, spreading growth being obtained in 24 hours. Glucose, sucrose and maltose are fermented, but lactose is not attacked. Gelatin is not liquefied and neither indol nor ammonia is produced.

The branching forms correspond with the *Streptothrix buccalis* described by Goadby. Eight strains were studied. All gave a hard, brown leathery growth which stuck fast to the

medium. Microscopically they are long, irregular, branching threads, the branches usually being almost at right angles to the main thread. They reacted variably to the Gram-stain, fermented glucose slowly, but failed to attack lactose or sucrose. Gelatin was usually liquefied. Indol was not produced.

In one instance a yeast and in another an oidium-like organism was isolated, but these forms are not significant and need not be described here.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. *Cladothrix placoides.* A 48-hour culture on glucose-agar, showing abundant growth and discrete white colonies.

Fig. 2. *Leptothrix buccalis. A.*—A 72-hour culture, kept about 9 months on artificial media, showing abundant growth on ascitic-fluid-agar. *B.*—A recently isolated culture showing only deep colonies in ascitic-fluid-agar. *C.*—A recent culture on ascitic-fluid-agar, showing better growth in stab than on surface.

Fig. 3. *B. acidophilus.* A 24-hour slant-culture on glucose-agar, showing colon-like rods. Stained with gentian violet.

Fig. 4. *B. acidophilus.* A 72-hour stab-culture in glucose-agar, showing pleomorphic rods. Stained with gentian violet.

Fig. 5. *B. putrificus.* A 48-hour culture, showing different stages of spore-formation and club-end spore. Stained with carbol-fuchsin.

PLATE 2

Fig. 6. *Cladothrix placoides.* A 72-hour culture, showing club-end threads, rods and coccoidal spores. Stained with methylene blue.

Fig. 7. *Cladothrix placoides.* A 48-hour culture stained with gentian violet.

Fig. 8. *Leptothrix buccalis.* A 48-hour culture, showing long, thick, club-end, tapering threads; some fragmented. Stained with gentian violet.

Fig. 9. *Leptothrix buccalis.* An older culture stained with carbol-fuchsin, showing faintly stained threads with heavily stained granules.

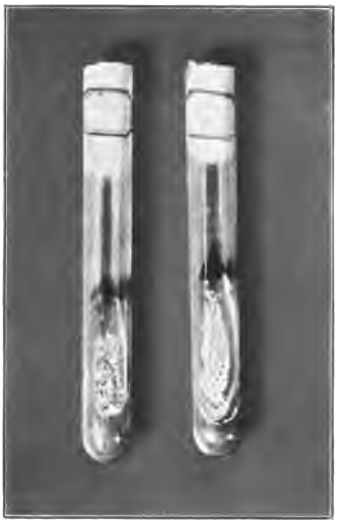

Fig. 1

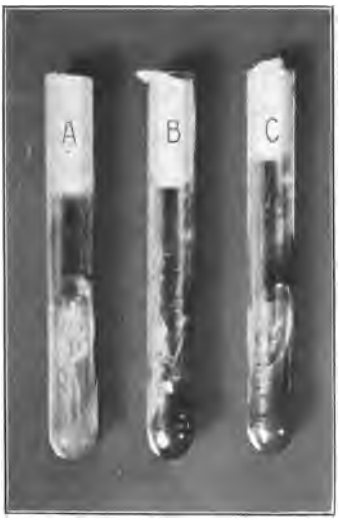

Fig. 2

Fig. 3

Fig. 4

Fig. 5

(See explanation on page 330)

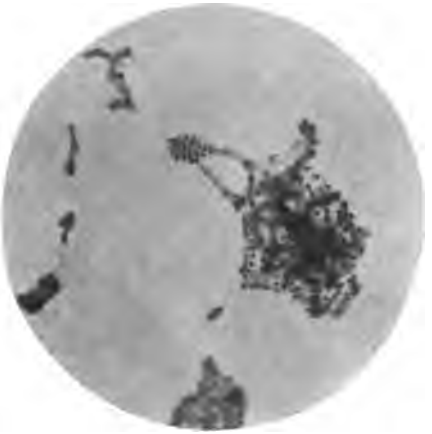

Fig. 6

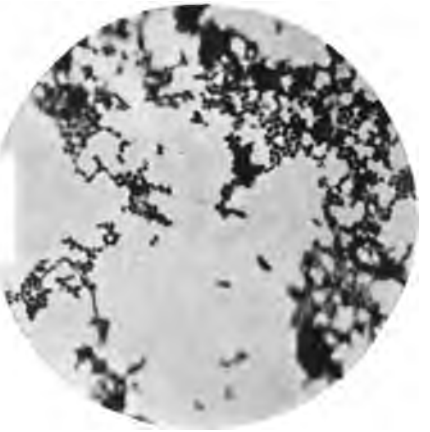

Fig. 7

Fig. 8

Fig. 9

(See explanation on page 330)

[Reprinted from the December, 1915, issue of THE JOURNAL OF THE ALLIED DENTAL SOCIETIES.]

CHEMICAL STUDIES OF THE RELATIONS OF ORAL MICROÖRGANISMS TO DENTAL CARIES.[1]

BY WILLIAM J. GIES AND COLLABORATORS.[2]

4. A Biochemical Study and Differentiation of Oral Bacteria, with Special Reference to Dental Caries (continued). (III)[3]

BY I. J. KLIGLER.

(*From the Biochemical Laboratory of Columbia University, at the College of Physicians and Surgeons, New York.*)

II. EXPERIMENTAL (continued).

(Second section.)

7. Biochemical data. In accord with the general plan outlined in the first paper of this series, and as initial steps toward the solution of the problems there suggested, experiments were made to ascertain (a) the degrees of fermentation of certain carbohydrates by oral bacteria; (b) the effect of the important salivary constituent—mucinate—on the growth of oral bacteria; (c) the action of oral bacteria on salivary mucinate; and (d) the

[1] Reports of findings in investigations conducted under the auspices of the First District Dental Society of the State of New York.

[2] This is the fourth section of the senior author's report for 1914-'15. See the *Journal of the Allied Dental Societies*, 1915, x, pp. 137, 141, 282. The fifth and sixth sections follow: *Ibid.*, pp. 459, 464. The next issue of the *Journal of the Allied Dental Societies* will present the delayed conclusion of the report for 1913-'14, to which reference was made in the second footnotes in the first and third papers in this particular series.

[3] Accepted by the executive officer of the Department of Biological Chemistry of Columbia University, as *Part III* (conclusion) of a dissertation, submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy, in the Faculty of Pure Science, Columbia University, June, 1915.

The strictly bacteriological part of the work was conducted in the laboratory of the Department of Public Health, American Museum of Natural History, under the supervision of the executive officer of the Biochemical Department of Columbia University. At Dr. Gies's request we were privileged to obtain, at the Clinic of the New York College of Dental and Oral Surgery, numerous specimens of dental deposits. We are indebted to Drs. Louise C. Ball and Frank L. Chambers for courteous and very helpful coöperation in this connection.

solvent action of certain oral microörganisms singly, and in combination, on powdered tooth in a glucose-containing medium.

FERMENTATION OF CARBOHYDRATES. The readiness with which glucose, lactose, and sucrose are fermented by oral bacteria is shown by the data in Table XXI, which relate to fermentation in standard sugar-free "meat-infusion" media,* containing the individual sugars (1 per cent) to be tested.

TABLE XXI.—DATA PERTAINING TO THE FERMENTATION OF SUGARS BY ORAL BACTERIA.

Sugars fermented	Glucose	Lactose	Sucrose	Glucose only	Glucose; also lactose	Glucose; also sucrose	Glucose, lactose, and sucrose individually
No. of bacterial strains tested..	426	386	326	426	386	326	322
Percentage of the strains that induced fermentation.....	99.5	70	72	15	23	16	56

* The standard media were made according to directions in a report to the American Public Health Association in 1912: *Standard Methods for the Examination of Water and Sewage*, p. 77; 1912. See text-books in bacteriology.

From the figures in Table XXI it is evident that glucose was fermented by practically all the strains represented in the available dental deposits. Lactose and sucrose were individually attacked less regularly though by large majorities of the strains. The data also show that the three sugars were attacked individually by just about half the number of strains, while the remaining strains fermented either glucose only or two of the three sugars individually.

Attempts were made to determine the relative fermentability of certain sugars in equivalent volumes, by typical oral bacteria, as measured by the resultant amounts of acid. The test media were prepared and the cultures inoculated by the junior author; the titrations for acidity were made in this laboratory by Misses Lottie M. Hull and Jeannette C. Mullikin under the senior au-

thor's supervision, and with the coöperation of Dr. Benjamin Horowitz.

The test media were made from "meat infusions" according to the standard methods[4] and included the addition of one per cent of the sugar to be tested. The tubes were sterilized for 30 minutes, in an Arnold steam sterilizer, on three successive days. The titrations were made with *n*/20 sodium hydroxid solution, phenolthalein serving as the indicator.

The results are expressed, in Table XXII, in terms of the number of cubic centimeters of normal hydroxid solution necessary to neutralize 100 cc. of the culture medium, each value representing average acidity produced by the different representatives of a given species, under approximately equal conditions of incubation for each form.

TABLE XXII.—DATA PERTAINING TO THE AMOUNTS OF ACID PRODUCED BY ORAL BACTERIA IN MEDIA CONTAINING DIFFERENT SUGARS.

Type of Organism	Sugar			
	Glucose	Sucrose	Maltose	Lactose
D. flavus	2.5	2.6	2.7	0.6
Staphylococcus	4.5	2.5	3.1	4.5
Streptococcus	4.2	4.2	...	4.0
B. acidophilus	5.6	0.9	5.4	5.8
C. placoides	3.2	4.3	3.7	0.5
L. buccalis	3.9	0.5*	2.7	0.4
Actinomyces	1.9	0.2	0.4	0.6

* This result is doubtful, the titration result for only one of the strains of this type having been recorded. Other representatives of this species actively fermented sucrose.

Another series of titrations were conducted, by the junior author, on lactose-broth media, to determine the relative amounts of acid produced by a number of recently isolated strains of staphylococci, streptococci and *B. acidophilus*. These types were selected because they represent the most active acid-producers. The average results were (in accord with the notation in Table

[4] The "standard methods" referred to here and elsewhere in this paper were described in a report to the American Public Health Association, in 1912: *Standard Methods for the Examination of Water and Sewage*, p. 77; 1912.

XXII): Staphylococcus, 4.8; streptococcus, 4.8; and *B. acidophilus*, 7.0.

From the data in Table XXII it is evident that glucose and maltose were more readily fermented, in general, than sucrose and lactose; also, that the amounts of acid produced, from the sugars used, were fairly constant in most cases for each type of bacteria. From the data in Table XXII, and those for the tests in lactose-broth media, it is apparent that *B. acidophilus* is capable of elaborating and withstanding a greater amount of acid than that produced and resisted by any of the other types.

THE EFFECT OF MUCINATE ON THE GROWTH OF ORAL BACTERIA. Pure sodium (salivary) mucinate, from supplies prepared for some of the senior author's studies in other relations, was added to standard media in a concentration of 2:1000 (0.2 per cent). Comparisons were made between growth in plain standard broth and that in broth containing mucinate; also between growth on mucin-agar and that on glucose-agar.

Over 60 strains, representing different oral types, were tested. Of these, 20 grew better on glucose-*agar*, 20 grew better on mucinate-agar, while 22 grew equally well on both media. Glucose was distinctly favorable for the growth of the *B. acidophilus*, while mucinate was decidedly stimulating to the growth of the thread-forms.

In the *broth*, 47 cultures grew equally well; 3 grew better in plain broth, while 15 grew better in the medium that contained mucinate.

Salivary mucinate evidently favors the development of specific oral bacteria (*C. placoides, L. buccalis*), but is without influence on a number that are not specifically oral types.[5]

We endeavored to ascertain the influence of salivary mucinate on the acid-producing power of oral microörganisms in glucose-containing media. Standard glucose-broth was prepared, divided into two portions and sodium mucinate added to one (0.2 per cent); the two media were then tubed and sterilized

[5] In the preparation of the culture media, the mucinate was subjected to temperatures intended to effect sterilization. It is possible, of course, that the influence of mucinate was increased or decreased by the heating process. We have no information on this point, however. The well-known resistance of glycoproteins in this relation suggests that the sterilization process was devoid of the suspected effects.

under uniform conditions. Ten cultures, chosen at random, were inoculated into each of the two media, the tubes incubated at 37 degrees C. for five days, and the ensuing acidity determined with *n*/20 sodium hydroxid solution, phenolthalein serving as the indicator. The results, shown in Table XXIII, indicate that the amounts of acid produced by the various organisms were approximately equal in both media.

TABLE XXIII.—DATA PERTAINING TO THE INFLUENCE OF SALIVARY MUCINATE ON THE ACID-PRODUCING POWERS OF ORAL MICROÖRGANISMS.

Number of culture	Mucin-glucose broth*	Glucose broth*
31a	3.2	3.3
48a	2.8	2.8
54	2.4	2.3
84	4.2	4.4
103	3.5	2.6
124	3.0	3.0
129a	3.5	4.5
180a	3.9	2.8
183	2.6	3.0
189	3.2	3.2

* The numbers represent acidity in terms of cubic centimeters of normal alkali solution required to neutralize 100 cc. of the culture.

THE ACTION OF ORAL BACTERIA ON SALIVARY MUCINATE. The influence of bacteria on the mucinate *in saliva* was not determined satisfactorily, in the few experiments we have tried thus far in this connection. Efforts to sterilize saliva, without removing or modifying the contained mucinate, were unsuccessful. Thus, passage of saliva through Berkefeld filters did not wholly prevent the appearance of bacteria in the filtrate, although it resulted in removal of most of the mucinate. The filtration was very slow and it is possible there was time enough for bacteria to "grow through" the filter into the filtrate. Further attention will be given to this matter in a succeeding report on this and related subjects.

The following method was finally adopted as the most desirable at this stage of our progress: Saliva was filtered through

a Berkefeld filter into a sterile weighed flask. The flask and saliva were then weighed, and powdered sodium mucinate added (0.5 per cent). By means of a sterile pipette, 10 per cent sodium carbonate solution was added, drop by drop, until the last portion of the mucinate, after thorough mixture, went into solution. This generally gave a medium that was neutral or slightly alkaline to phenolthalein. (The sodium mucinate was slightly acid to phenolthalein). The solution was then shaken with toluene, tubed with aseptic precautions into sterile tubes, and the latter heated on a water-bath for 30 minutes, on three successive days, at 60 degrees C. The tubes were then incubated and those that showed growth discarded. This treatment left the mucinate practically unaltered chemically (as shown by precipitation and reduction tests), drove out the toluene, and gave a fair number of sterile tubes.

Pure cultures of a number of oral microörganisms were inoculated into this sterile, artificial, saliva. The results were negative, that is to say, there was no increase in acidity and the amount of mucinate was undiminished, as determined approximately by comparisons of the masses of precipitates obtained after uniform acidification. These observations will be extended and the results described in the next annual report.

THE RELATIVE SOLVENT ACTION OF DIFFERENT TYPES OF ORAL BACTERIA, SINGLY AND IN COMBINATION, ON POWDERED TEETH IN A MEDIUM CONTAINING CARBOHYDRATE. The following method was employed in the tests in this connection: One-tenth gram portions of powdered human tooth were carefully weighed into clean, dry, Erlenmeyer flasks.[6] One hundred cc. of standard 1 per cent glucose-broth were then added to each flask and all were sterilized for 30 minutes, in an Arnold steam sterilizer, on three successive days. A small, platinum, loop-full of the tested culture was then inoculated into one of the flasks. Where a mixture of organisms was used, a loop-full of each culture was added to the contents of the flask. Into one flask (b)

[6] The powder was prepared from miscellaneous enamel and dentin fragments from normal teeth. The material was taken from a supply that had been used in the work discussed in the concluding section of the senior author's report for 1913-'14. (See footnote 2, p. 445.)

5 cc., into another (c) 7.5 cc., of normal lactic acid solution were placed, while one flask (a) was left free from lactic acid to serve as a non-acid control. The flasks were then incubated at body temperature for 15 days.

At the end of the incubation period, 5 cc. portions of the culture fluid from each flask were withdrawn to evaporation dishes, 45 cc. of distilled water added to each, and the acid contents determined with *n*/20 sodium hydroxid solution, phenolthalein serving as the indicator.

The cultures were next passed through fine filter-paper until perfectly clear filtrates were obtained. Two 40 cc. portions from each filtrate were then carefully pipetted into each of two small porcelain crucibles and the fluid evaporated to dryness on a water-bath. The residue was dried in a hot-air oven over night, and incinerated to constant weight in a "multiple unit" electric muffle. The degrees of solvent action of the organisms on the powdered tooth were ascertained by determinations of the amounts of calcium, in the resultant masses of ash, by the McCrudden method.[7]

The results for the types, or combination of types, of bacteria tested, in terms of the amounts of acid produced, and of the number of milligrams of calcium dissolved in 100 cc. of the filtered media, are summarized in Table XXIV. It is noticeable that there was lack of parallelism between the figures for total acidity and for the quantities of calcium dissolved, although in a general way a large yield of acid was associated with marked solution of calcium. Thus, No. 143 *(B. acidophilus)* brought about a solution of approximately the same amount of calcium whether grown separately or in combination with *B. putrificus,* even though the total acidity was different in the two instances. The same was true in the case of No. 54. In all the combinations in which it was tried practically the same amount of calcium went into solution, although the total acidity varied from 5.4 to 7.6.

The observed disparities were probably due to differences in the character, proportions and conditions of the acidic fermentation products; possibly, also, to undetected errors of analysis.[8]

[7] McCrudden: *Journal of Biological Chemistry,* 1911, x, p. 187.

[8] See the succeeding section of this report for further allusions to this matter: *Journal of the Allied Dental Societies,* 1915, x, p. 459.

TABLE XXIV.—DATA PERTAINING TO THE SOLVENT ACTION OF CERTAIN TYPES OF ORAL BACTERIA ON POWDERED NORMAL HUMAN TOOTH IN GLUCOSE-BROTH.

Organism	No. of culture	Acidity expressed as cc. of normal acid per 100 cc. of the culture	Amount of calcium in 20 cc. of the culture mg.	Average amount of calcium in 100 cc. of the culture mg.
B. putrificus.....	13	4.0	0.8800 0.9265	4.5160*
B. putrificus and *B. acidophilus*.....	13 143	6.3	3.4281	17.1405
B. acidophilus.....	143	7.3	3.3354 3.2428	16.4455
B. acidophilus.....	23	5.5	3.8450 4.0303	19.6885
C. placoides.....	54	5.4	4.9105 4.7252	24.0895
L. buccalis.....	124	3.4	0.9265	4.6325*
C. placoides and *L. buccalis*.....	54 124	6.4	4.2619	21.3095
B. acidophilus, *C. placoides* and *L. buccalis*.....	23 54 124	7.6	4.7252	23.6260
Streptococcus.....	177	4.1	1.8530 1.7604	9.0335
Staphylococcus.....	87a	3.6	1.2971 1.3898	6.7175
Controls: †				
Lactic acid solution, none.....	a	1.8	0.7412	3.7060
Lactic acid solution, 5 cc.....	b	5.0§	3.7913	18.9565
Lactic acid solution, 7.5 cc.....	c	7.5§	4.2546 4.0693	20.8100

* Practically the same as that for lactic-acid *control* "*a*".

† The lactic acid was a *normal* solution.

§ These values were obtained by direct titration. Judging from the result for "control a," the added lactic acid solution was not exactly *normal*.

Repetition and extension of these experiments will be made and the results discussed, in the next annual report, in the light of more numerous findings.

The results of these tests that appear to be particularly significant are (a) the lack of decalcifying power by the *B. putrificus,* which Rodella regarded as the etiologic agent in primary decay, also by the *L. buccalis,* a specific mouth form; (b) the relatively slight solvent action of both the streptococcus, considered by a number of modern investigators (Goadby, Baumgartner, *et al.*) as the cause of primary caries, and by the staphylococcus; (c) the marked decalcifying action of the *B. acidophilus* and the *C. placoides,* respectively, especially the latter.

The chemical data of these tests also supplement, in a striking way, the bacteriological findings. The organisms associated with, and predominating in, primary caries ferment the common sugars, readily produce a high degree of acidity, and bring about considerable dissolution of tooth substance. On the other hand, organisms normally abundant in the mouth but not directly related to primary enamel decay, as well as those that are prominent in the later stages of caries, do not produce a high degree of acidity and induce but slight dissolution of powdered tooth.

III. SUMMARY OF BACTERIOLOGICAL AND CHEMICAL FINDINGS.[9]

Material collected from the surfaces and cavities of teeth, in 40 individuals, was studied with the object of determining the numbers and types of bacteria in such deposits, normally and at various stages of decay. Briefly summarized, the results were as follows:

1. Under ordinary conditions the number of bacteria *in a milligram of deposit* on normal teeth, and cultivable on nutrient agar-plates, was about 1,000,000; when estimated with the microscope, 25,000,000. In "dirty" mouths the counts for dental deposits were about twice as high. The numbers of bacteria on unbrushed teeth were about four times as great as those on brushed teeth, while the count for normal dental deposits obtained immediately after meals was increased about three times that before the meal. In the first stages of caries, the numbers of bacteria in material from enamel cavities were 100 millions or more, whereas in similar material from cavities involving decayed

[9] This summary relates to papers 2, 3 and 4 of this series (sections 2, 3 and 4, respectively, of the annual report for 1914-'15, by the senior author, to the First District Dental Society of the State of New York.) See footnote 2, p. 445.

pulp there were decided decreases, the numbers falling to about 40 millions per milligram.

2. Qualitatively, the types of bacteria most prevalent in deposits on normal teeth were the cocci, which represented about 75 per cent of the total flora; 40 per cent of the cocci were streptococci. In the primary stage of caries the relative abundance of types was different from that in deposits on normal teeth; only 40-50 per cent of the forms were cocci, while the percentage of thread-forms rose to about 30 per cent (an increase of about 200 per cent), accompanied by a correspondingly large increase in the number of the non-spore-forming, actively acid-producing rods. In decay of the pulp, on the other hand, the cocci remained low in proportion, the thread-forms almost disappeared, the non-spore-forming rods continued to be quite as numerous as they were in the primary stages of caries, but a new form—an anerobic, putrefactive, spore-bearing rod—was found in large numbers.

3. From the chemical standpoint, almost all these organisms are capable of fermenting glucose and maltose; a majority of them also ferment either lactose or sucrose, or both. The cocci usually ferment each of these four sugars, and produce, thereby, moderate degrees of acidity. The non-spore-forming rods ferment glucose, maltose and lactose, with high degrees of acid-production, but usually fail to act on sucrose. The thread-forms, on the other hand, ferment glucose, maltose and sucrose but not lactose. The short-thread form (*C. placoides*) produces considerable quantities of acid and has the property of clinging to smooth surfaces. The putrefactive variety ferments sugars, but in their absence is highly proteolytic, digesting serum, casein and gelatin very rapidly, under anerobic conditions.

4. Salivary mucinate acted as a stimulant (nutrient?) for the growth of the leptothrix type, but had no appreciable effect on other forms. There was no appreciable action of the bacteria on sodium mucinate in the few experiments conducted in this connection.

5. The bacterial types usually found in the deposits on normal teeth exerted but slight solvent action on powdered tooth in 1 per cent glucose-broth, whereas the types that prevailed in

cavities in the first stages of enamel decay brought about marked dissolution of the powder. Putrefactive organisms from decayed pulp were devoid of solvent action on powdered tooth in glucose-broth.

IV. Concluding discussion.

The results of the various experiments described in this report exhibit marked unity, which becomes more apparent when we consider the nature of, and the effect of certain environmental factors on, bacterial activity. Under a given set of imposed conditions bacteria tend to behave in a certain definite and constant manner. In a pure culture it is a relatively simple matter to ascertain the reactions between a given organism and a particular environment. Under conditions as they exist in nature, however, where organisms live in groups of families and communities [what Marshall (2) calls microbial associations], there are other factors to consider, *e.g.*, shifting environment, effects of various types of bacteria on one another, and special modifications of the environment itself by one or more of the existing types that render it favorable or unfavorable to the others. For example, *B. putrificus* is a strict anerobe and alone will not grow in the presence of free oxygen. In association with an aerobic form, however, it grows readily even in the presence of free oxygen. Under natural conditions there is, furthermore, a succession of types due to shifting of the environment. This is prominently brought out and taken advantage of in the purification of sewage, where, with a change from an anerobic to an aerobic environment, flora may be transformed from a putrefactive to an oxidative character.

The effect of environment as a selective agent, has been especially noted for the intestinal flora of man and animals, and has recently been brought under experimental control. Thus, Herter and Kendall (1) transformed the flora of monkeys and cats by feeding either a carbohydrate or a protein diet. In the former instance an acidific, in the latter a proteolytic, flora was established in the tract. Last year Rettger (3, 4) and his associates obtained similar results with white rats and chickens. Torrey (5) was able to transform the flora of typhoid patients

by means of specific diets. In all these experiments carbohydrate feeding promptly brought about a preponderance of the acidophilic types. In all these instances the balance was merely shifted, the power of one type of bacteria having been enhanced, that of another type suppressed. Under no condition, apparently, was either type completely eradicated.

These observations have a definite bearing on the conditions in the mouth and the factors influencing decay. The mouth is in many respects similar to the intestinal tract. Like the tract, it is sterile at birth and, thereafter, constantly receives a large and multifarious supply of bacteria. Stagnant conditions characteristic of the intestine also exist in the mouth to some extent, during sleep particularly, but the mouth receives many forms that never survive passage through the stomach. Furthermore, the medium in the mouth is lymph-like and generally carbohydrate-containing, while that of the intestine is highly nitrogenous. In consequence of this environmental difference, the characteristic flora of the normal mouth is one that produces acid to a moderate degree (cocci), while that of the intestine usually shows a dual nature —both fermentive and putrefactive (coli, proteus, etc.).

Stagnant conditions in the intestine effect concentration of proteins and consequent predominance of proteolytic flora. Stagnation in the mouth, on the other hand, induces concentration of fermentable carbohydrate. As a consequence, conditions in the mouth are similar to those in the intestine after ingestion of a carbohydrate diet. A corresponding change in the flora would be expected to follow such a condition. This is exactly what occurred in the case of the unbrushed teeth, where the acidific types began to assert themselves as a result of the stagnant condition in the mouth during the night. If, now, this stagnant condition were not temporary and general, but permanent and local, such as follows the lodgment of a particle of meat in a crevice in a tooth or in a space between teeth, the protein would remain practically unaltered by the saliva and by the bacteria which, excepting certain anerobic forms such as *B. putrificus,* have hardly any action on complex proteins in general. Starchy food, on the other hand, if localized against teeth, is hydrolyzed by salivary amylase and the resultant sugar is promptly converted by bacteria

into acid, leading to stimulation of the growth of highly acidific forms and to consequent suppression of the others. This condition was noted in the primary stages of caries, where the *B. acidophilus* came prominently to the fore, and explains why dental crevices and interspaces are favorable points for the initiation of caries.

On the whole, then, there are conditions and phenomena in the mouth that are analogous to those occurring in soil, in sewage purification, in the intestinal tract, and elsewhere, where associative bacterial activity exists, and where sudden and radical changes in the environment take place, either from the standpoint of the concentration of oxygen or the character of the nutrients.[10]

These observations support the suggestions and inferences by the senior author, in preceding annual reports, which led to the initiation of the studies of which this section of the report for 1914-'15 is a part.

V. Summary of general conclusions.

1. The flora of deposits on normal teeth was constant within certain limits. While the absolute numbers fluctuated with the condition of the mouth, teeth, etc., the relative abundance of the different types remained approximately the same.

2. The stagnant condition in the mouth during the night, and the ingestion of food, caused marked increase in the number of bacteria in deposits on normal teeth, in the former instance shifting the relative abundance of types. The results indicate, in accord with prevailing opinion in regard to oral hygiene, that washing the teeth before retiring is very desirable, and that rinsing the mouth after each meal is just as expedient a habit to cultivate as washing the hands before a meal.

3. The early stages of caries are characterized by a decided alteration in the relative abundance of types as they occur in deposits on normal teeth. Three forms, the *B. acidophilus*, the *C. placoides* and the *L. buccalis*, were prominent in the carious enamel deposits.

[10] See pp. 312-314 of the third paper in this series: *Journal of the Allied Dental Societies*, 1915, x.

4. In pulp decay an anerobic, spore-bearing, putrefactive bacillus, *B. putrificus,* was always prominent.

5. The organisms prevalent in primary enamel decay very actively ferment the common sugars and bring about comparatively great dissolution of powdered tooth. The organisms in deposits on normal teeth and in the later stages of caries exert either slight effects, or none at all, in these relations.

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* Additional items in the bibliography of this series (2, 3, 4) appear on pages 165 and 329 of this volume.

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